INVESTIGATION OF DRUG IONIC LIQUID SALTS FOR TOPICAL DELIVERY SYSTEMS

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Abstract

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Revealing ionic liquid drug salts for topical delivery systems

Key words: Ionic liquids (ILs), poorly water soluble drugs, topical drug delivery, partitioning, ion-pairing, carbomer, oligomer, benzalkonium ILs, ILs with mixed anions, hydrogel, NSAIDs, sulfacetamide, pharmaceutical performance,

Pharmaceutical companies and FDA (Federal Drug Administration) rules rely heavily on crystalline active pharmaceutical ingredients delivered as tablets and powders in the form of neutral compounds, salts and solvates of neutral compounds and salts. About half of all drugs sold in the market are in the form of salts which are held together by ionic bonds along with some other forces. Recently, Ionic liquids (ILs) an interesting class of chemical compounds have offered potential opportunity for exploration as novel drug ionic liquid salts, particularly in the field of transdermal/topical drug delivery. Due to the multifunctional nature of these salts they could allow generation of new pathway to manipulate the transport and deposition behaviour of the drug molecule. It is this modular approach of IL that forms the basis of the research presented here, in which pharmaceutically acceptable compounds are combined with selected drugs with known problems.

IL salts were generated by combining at least one drug molecule with FDA approved compounds and were assessed for physicochemical properties, skin deposition and permeation studies. Skin deposition data suggested that these systems exhibit high skin retention, which was found to correlate with the

molecular weight. On the other hand, permeation data displayed an inverse relationship between flux values and molecular weight of the permeant. Similar work was extended with ILs with mixed anions containing two drugs. The benzalkonium-sulfacetamide ILs were investigated for synergism and the biological studies data display no synergistic effect. It was also illustrated that in-situ IL based ibuprofen hydrogels systems could be manipulated via IL approach for topical application. These findings suggest the potential applicability of IL based formulations for topical delivery of drugs.

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I would like to dedicate this thesis to my parents, my wife and family members, who have been giving me unconditional love and care, for everything they have done for me.

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Abbreviation

RTIL	Room temperature ionic liquid
API	Active pharmaceutical ingredient
FDA	Federal drug administration
NSAID	Non-steroidal anti-inflammatory drug
NMR	Nuclear magnetic resonance spectroscopy
TGA	Thermal gravimetric analysis
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy
HPLC	High performance liquid chromatography
[BTEA][Ibu]	Benzyltriethylammonium ibuprofen
[BTEA][Diclo]	Benzyltriethylammonium diclofenac
[BTEA][ISulfa]	Benzyltriethylammonium sulfacetamide
[BTEA][Sal]	Benzyltriethylammonium salicylate
[BDMA][Ibu]	Benzyldimethylhexadecylammonium ibuprofen
[BDMA][Diclo]	Benzyldimethylhexadecylammonium diclofenac
[BDMA][Sulfa]	Benzyldimethylhexadecylammonium sulfacetamide
[BDMA][sal]	Benzyldimethylhexadecylammonium salicylate
[BTEA-Cl]	Benzyltriethylammonium chloride
[BDMA-Cl]	Benzyldimethylhexadecylammonium chloride
[Sod-Sulfa]	Sodium salt of sulfacetamide
S.aureus	Staphylococcus aureus
E.coli	Escherichia coli
OD	Optical density
%GI	Percentage growth inhibition

Ko/w	Octanol-water partition coefficient
Td	Degradation temperature
Tc	Crystallisation temperature
Tg	Glass transition temperature
Tm	Melting temperature
µg/ml	microgram per millilitre
s	Singlet
d	Doublet
t	triplet
q	Quartet
m	Multiplet
br	Broad
SEDDS	Self emulsifying drug delivery systems
IC50	Half maximal inhibitory concentration
EC50	Half maximal effective concentration
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration

Chapter 1 Introduction

Solid materials exist as single or multicomponent crystals or amorphous forms. Crystal engineering involves tailoring of properties of the solid materials to achieve desired product properties. The pharmaceutical industry relies on crystalline active pharmaceutical ingredients (APIs), which can be found in several forms including salts, hydrates, solvates, crystalline and amorphous materials and co-crystals. The solid APIs (BCS Class II) suffer from several problems such as low solubility, low bioavailability and polymorphism. However apart from the issues mentioned above there are number of other factors such as morphology, particle size and bulk properties which could have significant effect on the pharmacokinetics of the solid APIs. The main challenge of the pharmaceutical industry in drug development is to improve the bioavailability and low solubility of new and currently marketed drugs which still remains the same (Shamshina and Rogers 2014). Co-crystals are emerging as an attractive alternative to these solid APIs. Co-crystals can enhance the physicochemical property of the solid APIs such as hygroscopicity, solubility and compaction behaviour without affecting the pharmacological behaviour of APIs (Weyna et al. 2009; Khan et al. 2010). However co-crystals also suffer with the same problems which are associated with solid crystalline active pharmaceutical ingredients comprising polymorphism (Karpinski 2006).

Recently Ionic liquids (ILs) have been growing in interest within various pharmaceutical fields including the formulation of active pharmaceutical ingredient ILs (API-ILs). Ionic liquids are defined as organic salts that are liquid below 100 °C. The generation of ILs involves the reduction of melting point of the APIs leading to an increase in solubility and dissolution rate (Hough et al.

2007a; Stoimenovski et al. 2010a). More importantly, ILs can be generated with tailored properties for any specific application. The physicochemical properties such as viscosity, hydrophobicity, solubility and density can be fine-tuned by simply choosing combinations of cations and anions. The molar ratio of counterions and degree of ionocity are vital properties for pharmaceutical companies as these factors are responsible for governing bioavailability of active pharmaceutical ingredients including solubility, absorption, distribution, metabolism and finally excretion (Kelley et al. 2013). ILs have been reported as carrier for drug delivery (Moniruzzaman et al. 2010b) and as drug ionic liquid salt for topical/transdermal drug delivery (Park and Prausnitz 2015). ILs have been used as additives in several topical formulations (Kamal et al. 2006; Kamal et al. 2007; Moniruzzaman et al. 2010b; Bica et al. 2011; Dobler et al. 2013; Goindi et al. 2014).

Nowadays, the topical drug delivery pathways (transdermal, buccal, nasal, vaginal, rectal, dermal, ocular etc.) have an increasing part in pharmaceutics. The vital reason for the importance of topical application of drugs is not only the elimination of first-pass metabolism leading to enhanced bioavailability but also a reduction in side effects caused by the decline of the drug concentration after orally administered drugs. Apart from this many beneficial advantages are offered by topical delivery of drugs, such as patient-tailored delivery, extended duration of activity and avoidance of several dosing schedules (Cevc 1997). Topical and transdermal drug delivery is associated with three basic target sites which are (a) skin surface (b) the skin itself (epidermis and dermis) (c) systematic circulation. The skin surface is the prime target when considering cosmetics, insect repellent and disinfectants. Topical drug delivery is basically targeting various layers of skin where the disease state is present within the

organ itself, for instance in inflammatory disorders and microbial infections of the skin. On the other hand, systemic circulation is the target site for transdermal drug delivery (Morrow et al. 2007). The prime issue associated with topical drug delivery is that only a limited number of APIs have the ability to overcome the biological barrier in the body. Skin is the well-known biological barrier to humans, the topical and transdermal delivery of APIs becomes very difficult and in some cases impossible because of the very highly ordered structure and diffusional resistance properties of the outermost layer of the skin which is called as stratum corneum (SC) (Schäfer-Korting et al. 2007). Development of drug delivery through skin is an innovative and important research area in modern therapy. The drug molecule should possess enough solubility; it should not only display adequate lipophilic nature to penetrate via the SC domain but should also possess sufficient hydrophilic property to distribute in the tissues of the epidermis (Benson 2005).

Some conventional non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and diclofenac have been reported to increase cardiovascular risk (Komatsu and Sakurada 2012)**.** When administered orally, ibuprofen is largely metabolized in the liver resulting into a short biological half-life. That is why ibuprofen administration frequently causes extensive gastric ulceration when it is used for long duration especially in the case of treatment of arthritis. Ibuprofen is widely used for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and is been rated as the safest traditional NSAIDs(Bushra and Aslam 2010). The oral administration of diclofenac is also associated with severe gastrointestinal, cardiovascular and renal side effects. The mechanism of action of diclofenac is via inhibition of prostaglandin synthesis (Altman et al. 2015). Diclofenac is a poorly water soluble drug

because of its high melting point and intrinsic hydrophobicity(Cordero et al. 1997).

When seeking alternatives to oral administration, properties of other materials relevant to topical administration should be considered. Salicylic acid is found to possess a broad range properties such as anti-inflammatory, analgesic, antiseptic and preservative, and a key ingredients of many skin care products (Wu 2007). Sulfacetamide is a sulfonamide antibiotic drug which is prescribed in the form of sodium salt and finds its applications in ophthalmic (Sridhar et al. 2001) and skin (Margolis et al. 2005). Sulfonamide drugs destroy bacteria by blocking the synthesis of folic acid in bacteria (Maren 1976). Topical application of ibuprofen, diclofenac, salicylic acid and sulfacetamide in the form of an IL drug salt not only allows elimination of the solid state problems but also helps to achieve higher local concentration of the drug at the site of inflammation and pain (Tegeder et al. 1999; Reddy et al. 2011).

It is a challenging task for pharmaceutical scientists to develop efficient topical drug delivery systems with enhanced penetration effects for API absorption. Due to recent advances in the field of ILs including pharmaceutical applications, the fact that ibuprofen, diclofenac and salicylic acid are considered as one of the most preferred NSAIDs for the treatment of arthritis and various inflammatory conditions and sulfacetamide is a common and useful antibiotic drug, this study aimed to investigate such ionic liquid drug salts for topical drug delivery systems.

1.1 Aim of the study

To synthesise pharmaceutically acceptable ILs based on ibuprofen, diclofenac salicylic acid, sulfacetamide and investigate these neat API-ILs as topical delivery systems.

1.2 Specific objectives

1. To examine effect of counterions on physicochemical properties and biopharmaceutical performance of benzalkonium based NSAIDs ILs.
2. To investigate synergism and the effect of cationic counterion benzalkonium on penetration behaviour of benzalkonium-sulfacetamide via Ionic liquid (IL) approach
3. To understand the effect of ionic/hydrogen bonding interaction: insights into the physicochemical properties and transport behaviour of benzalkonium based mixed anion ILs.
4. To consider the potential of In-situ formation of ibuprofen – IL in topical ibuprofen gel formulation.

1.3 Scope of thesis

On the basis of the physical, chemical and biological properties drug molecules can exist as neutral or salt forms (Stahl and Wermuth 2002). Approximately half of all the therapeutic drugs are used as solid salts. As already discussed, ILs have been studied as carriers for topical/transdermal drug delivery. In order to understand the drug ILs as topical drug delivery systems, this thesis will discuss the formation of ILs from pharmaceutically acceptable drug molecules and how the structure of cations and anions, and the interaction between them will affect the physicochemical properties and pharmaceutical performance of drug molecule.

Chapter 2 provides background information and a comprehensive literature review of papers relevant to this study. Chapter 3 describes the materials, general methods and the experimental techniques used to determine the physicochemical properties and characterisation of the synthesized ILs.

Results of the experimental work are then presented in Chapter 4 which explains the formation of ILs with benzalkonium cation with anions (ibuprofen, diclofenac) which have been already used but the pharmaceutical performance, specifically in regard to topical application, has not been reported. The effect of counterions on the physicochemical properties, skin deposition and permeation profiles was evaluated.

Chapter 5 illustrates the investigation into any synergistic effect of combining benzalkonium cation and the antibiotic sulfacetamide in anionic form. The effect of cationic counterion benzalkonium on the skin deposition and permeation profile of sulfacetamide along with the physicochemical properties wasalso studied.

The data obtained from Chapter 4 provides important information regarding the effect of counterions on the physical and chemical properties of the ILs. However, the ILs studied in Chapter 4 were for two component systems consisting of only one drug molecule therefore research regarding three component systems (ILs with mixed anion) containing two drug molecules was the prime focus of Chapter 6. The same benzalkonium cation used in the work described in the Chapter 4 was coupled with ibuprofen and salicylic acid to generate IL with mixed anion which was characterised by NMR spectroscopy. The combination of two drug molecule will not only provide dual functionality to the IL but also allows lowering of melting point. Thus, this chapter provides the insights of the physicochemical properties of ILs with mixed anions and the effect of ionic and hydrogen bonding interaction on the permeation rates of the drug molecules.

Chapter 7 is more focused on the understanding of the interactions between the ingredients during formulation development. The attention of this chapter is on

demonstration of in-situ formation of ibuprofen IL during formulation and penetration of ibuprofen via artificial membrane.

Finally, Chapter 8provides conclusions obtained from the study described in this thesis and delivers suggestions for future work.

Chapter 2 Background and Literature

2.1 Background

2.1.1 Introduction of ionic liquid

Ionic liquids (ILs) are defined generally as liquids composed entirely of ions with melting points or glass temperature below 100 ºC(Hough et al. 2007b). Those ILs which are liquid at room temperature are considered as room temperature ionic liquids (RTILs) (Ferraz et al. 2011) (Figure 2.1). ILs can be distinguished from molten/fused salts as ILs are generally composed of an organic cation instead of an inorganic cation. This avoids the disadvantages of molten/fused salts, such as their high working temperatures and their corrosive and viscous nature. The incorporation of asymmetric and bulky organic moiety leads to a decrease in melting temperature. Melting point of the derived ILs generally depends on the size and complexity of the ions. For example the melting point of sodium chloride is 803ºC, even though the melting point of the eutectic mixture of LiCl-KCl is 355 ºC whereas the imidazolium chloride displays 77-79 ºC (Wasserscheid and Welton 2008)

Figure 2.1 Representation of ionic liquid (Shamshina et al. 2015)

2.1.2 History of Ionic Liquid Research

The history of ILs can be traced back to the mid-19th century, when a chemist reported ‘red oil’ produced from a side product of a Friedel- Craft reaction. Later on a group of chemists in Japan suggested that the red oil was a mixture of alkylated aromatic ring cation and chloroaluminate anion and gave this the name ionic liquid (Dean et al. 2010a).

The very first known room temperature ionic liquid was synthesized by Walden in 1914,has a melting point of 12 ˚C and is known as ethyl ammonium nitrate (Plechkova and Seddon 2008) (Figure 2.2).

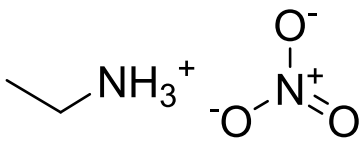


Figure 2.2 Ethyl ammonium nitrate

Soon after this work, ionic liquids gained enormous interest as a research field and this interest continued growing throughout and after the nineteenth century. Extensive research in ILs was carried out in 1960s and 1970s with the discovery of chloroaluminate salts for the use of thermal batteries which was discovered by US Air force Academy in collaboration with research groups at University of Colorado State. A number of ILs investigated during this time period were found to have low melting points but suffered with hydrolysis issues; this led to the search of more stable ILs (Koch et al. 1976)(Gale et al. 1978).

Investigation of Imidazolium cation based ILs with ions such as acetate, sulphate, tetrafluoroborate, hexafluorophosphate found them to be water stable at room temperature. The discovery of water and air stable ILs gained enormous interest in various fields (Wilkes and Zaworotko 1992). The alkylated

substituted imidazolium and pyridinium cations with halide anions are applied as electrolytes. There has been an enormous increase in research publications on ionic liquids as catalyst, electrolytes, solvent /co-solvents over the past two decades(Wilkes 2002).

2.1.3 Properties of Ionic Liquids

Ionic liquids have significant and interesting physicochemical properties. Research on their physical, biological and chemical properties is very limited when compared with traditional organic solvents. Recently this field has been of great interest due to the fact that the physical, chemical and biological properties of ionic liquids could be altered with specific requirements for various applications. This tunability of their physicochemical properties opens a new challenge in this research area. Quaternary ammonium salts have been available commercially for a long time and some are best known for their application as phase transfer catalysts. The most useful property of ionic liquids is the independent ‘tunability’ of the cations and anions, and this is the key feature of ionic liquids which gives them a wide range of properties. For example, miscibility and immiscibility with water, acidic, basic and neutral nature of ionic liquids and toxicity and nontoxicity. Over 200 known ionic liquids have been synthesized but for most of the ionic liquids, the data relating to their physicochemical properties is not available or is incomplete.

Generally they have negligible vapour pressure, high liquidus range, high thermal stability, low combustibility, high ionic conductivity, a large electrochemical window and the ability to solvate compounds of varying polarities. ILs are used as solvent to facilitate many important chemical reactions such as Diels Alder reaction and Friedal Crafts alkylation and acylation reaction (Brennecke and Maginn 2001). They can be used as acids,

bases, ligands and an important precursor to prepare stable carbenes. The solubility of different species in ILs depends on the basis of polarity and hydrogen bond.

2.1.3.1 Melting points

Ionic liquids are commonly defined as salts with a melting point below 100 ˚C. The components of ILs have a significant impact on the physicochemical properties. Both cations and anions, along with the charge distribution contribute to the melting point of the ILs (Wasserscheid and Welton 2008). The melting point of ionic liquid depends on the size of the cation: generally melting point decreases with increase in the size of the cation. For example phosphonium and tetraalkylammonium salts with alkyl shielded charge provide depressions in the melting point (Wasserscheid and Keim 2000). Symmetry of the cation also plays an important role, as ions with higher symmetry have higher melting points which are due to more efficient ion packing in the crystal unit cell. On the other hand unsymmetrical cations lead to delocalisation of charges in the crystal unit cell, which reduces lattice energy and finally leads to decrease in melting point (Gordon and Rao 1978).The melting temperature of the system could be decreased or increased by varying aromatic stacking, ion packing and methyl-pi interactions (Wasserscheid and Welton 2008). The melting temperature of 1-alkyl-3-methylimidazolium tetrafluoroborate system was studied as the function of alkyl chain length and the melting point of the system was found to decrease with increase in chain length due to disruption of the lattice unit cell. The increase in melting point was observed for the alkyl chain length greater than 10 carbon atoms because of the increase in Van der Waals interaction between alkyl chains which leads to formation of liquid crystalline phases (Holbrey and Seddon 1999). The melting point of the ILs with

higher degree of alkyl branching was found to be higher, which could be attributed to the efficient ion packing due to the decrease in the free rotation volume (Ngo et al. 2000).

The size and structure of the anion also plays a significant role in the melting point of ILs. Increase in the size of anion leads to decrease in melting point of ILs because of the disruption in the columbic interactions causing decrease in lattice energy (Wasserscheid and Keim 2000). Hydrogen bonding in the lattice is a major factor in increasing melting point. ILs with strongly coordinating anions such as halides exhibits higher melting point because of their ability to form hydrogen bonding. On the other hand ILs with highly fluorinated anions have lower melting point due to delocalisation of charge (Gordon et al. 1998; Hardacre et al. 2002).

2.1.3.2 Polarity

Polarity is an important property used to describe solvation capability of a solvent, when considering a solvent for a particular application. Several ILs based on 1-alkyl-3-methylimidazolium were tested for polarity using solvatochromic Nile Red. The visible absorption maximum wavelength was found to move towards longer wavelength when Nile Red was dissolved in the increasingly polar solvents. This study illustrates that the value of visible absorption maximum wavelength obtained from ILs was found to be similar to short chain alcohols (Carmichael and Seddon 2000).

Solvation in ILs occurs through a number of interactions such as pi-pi interactions, Van der Waal interactions, ionic interactions, dipole-dipole and hydrogen bonding. Hence it could be suggested that ILs exhibits greater solvating power when compared to traditional organic solvents (Freemantle 2010). However, the magnitude of the various interactions depends on the

components of ILs (cation and anion), substituted alkyl chain length and on the solute(Freemantle 2010).Dielectric constant or relative permittivity is used to determine the polarity of solvent. ILs are composed of ions therefore they are supposed to conduct electricity. However, indirect methods such as microwave dielectric spectroscopy could be used to measure the polarity of ionic liquids (Wakai et al. 2005).

2.1.3.3 Liquid range and thermal decomposition

Liquid range is also known as liquidus range, which is defined as the temperature between melting point or glass temperature and boiling point or thermal decomposition temperature. ILs has far higher liquid range than molecular solvents. The liquidus range of organic solvents is typically in the range of 100 to 200 k whereas ILs liquidus range falls in the region of 300 to 500 K. The higher liquidus range of ILs offers an opportunity to work at much higher temperature ranges which could not be achieved by using traditional organic solvents (Freemantle 2010).

Generally ILs are considered to be non-volatile therefore the higher limit of liquid range of ILs is governed by thermal decomposition temperature (Wasserscheid and Welton 2008). Research has shown that the degradation pathway of imidazolium based ILs was found to be the reverse of the reaction pathway used to generate ILs (Chan et al. 1977). The authors have investigated the thermal stability of imidazolium based ILs with different anions such as Cl,I, BF_4, DBS (para-dodecylbenzenesulfonate), PF_6, NTf_2. The stability of ILs was found to be inversely proportional to the stability of the alkyl-anion species formed through the decomposition process (Maton et al. 2013).

2.1.3.4 Conductivity

A large ionic conductivity was originally expected for ionic liquids, considering they are primarily comprised of ionic species. However this is not the case and the conductivity of ILs was found to be relatively low at room temperature (Noda and Watanabe 2000). The number of the charge carriers and their mobility is the primary factor responsible for the conductivity of any solution. The conductivity of ILs is far less than concentrated aqueous electrolytes because of the reduction of free charge carrying species due to the ion-pairing and the presence of large constituent ions which reduce ion mobility (Wasserscheid and Welton 2008).

ILs based on imidazolium cation exhibit higher conductivities (10 mS/cm) than ionic liquids based on dialkylpyrrolidinium, tetraalkylammonium, piperidinium and pyridinium which is in the range of 0.1 mS/cm to 5 mS/cm (Galiński et al. 2006).IL conductivity decreases with decrease in planarity of the cation, the flat imidazolium cation has a higher conductivity than the tetrahedral tetraalkylammonium cation while the pyrrolidinium based Ils have an intermediate conductivity (MacFarlane et al. 1999).

Temperature, viscosity and conductivity are strongly interrelated with each other. The conductivity increase with decrease in viscosity while increases with increase in temperature. For example the conductivity of 1-ethyl-3-methylimidazolium bis[(trifluoro-methyl)-sulfonyl] amide and 1-ethyl-3-methylimidazolium tetrafluoro borate with respect to temperature is shown in Figure 2.3 .

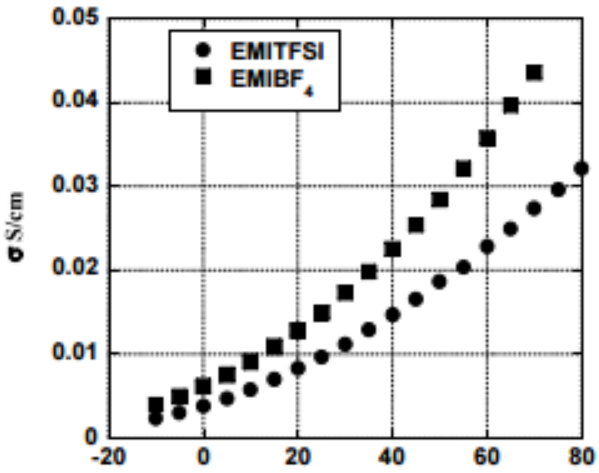


Figure 2.3 Variation of conductivity with temperature for EMITFSI and EMIBF4 (Garcia et al. 2004)

2.1.3.5 Vapour pressure

Generally ILs are reported to have negligible vapour pressure but this does not mean that all ILs have no vapour pressure. Negligible vapour pressure is one of the most celebrated properties of ILs as they do not evaporate in reaction vessels and cannot contribute to related health concerns or to air pollution. The strong coulombic interaction between the components of ILs is the significant factor responsible for the lack of vapour pressure at temperatures up to their thermal decomposition temperature. Using density and experimental surface tension dataa group of scientists reported that ILs could be designed to undergo distillation. Imidazolium cations containing long alkyl chain lengths with $[NTf_2]^-$ anions such as $[C_{10}mim]$ $[NTf_2]$ and $[C_{12}mim]$ $[NTf_2]$ could undergo distillation at temperatures between their estimated boiling and decomposition temperatures (Earle et al. 2006).

In general, the vapour pressure of ionic liquids, especially for the widely used imidazolium ionic liquids with short alkyl chains are negligible at room

temperatures and pressures. Consequently most of the ILs display very little or no evidence of distillation below their decomposition temperatures.

2.1.3.6 Solubility in ILs

ILs are generally considered to be polar solvents having polarities equivalent to alcohols and aprotic solvents such as: dimethylformamide and dimethylsulfoxide. ILs are also called as designer solvents, as they can be designed to be alternatives to organic solvents for variety of applications including synthesis, electrochemical processes and liquid/liquid separations (Carmichael and Seddon 2000)(Aki et al. 2001).

The polarity and co-ordinating ability of an IL is significantly affected by the components of the IL system and can be tailored for specific purposes. The lipophilicity of the system can be varied through cation substitution and the solubility of IL in water can be tuned from slightly miscible to complete miscible by changing the anion of the system from Cl^- to $[PF_6]^-$. The solvent property of the IL depends on the H-bond accepting, H-bond donating abilities of the components of the IL system (Wasserscheid and Welton 2008).

Authors have also investigated the interactions of ILs with organic solutes and suggested that ILs containing long alkyl chain can act as low polarity phases, which strongly interact with non-polar solutes. Polar solutes having proton donor functional groups interact strongly with ILs, while the solutes such as esters, ketones and aromatics with proton donor/acceptor functional groups interact through Van der Waals and ion-dipole interactions (Armstrong et al. 1999).

2.1.4 Classification of Ionic liquids

A. Classification on the basis of degree of ionization

Ionic liquids are organic salts which are entirely composed of ionized species with melting point below 100 ˚C. On the basis of degree of ionization ILs are classified into the following species.

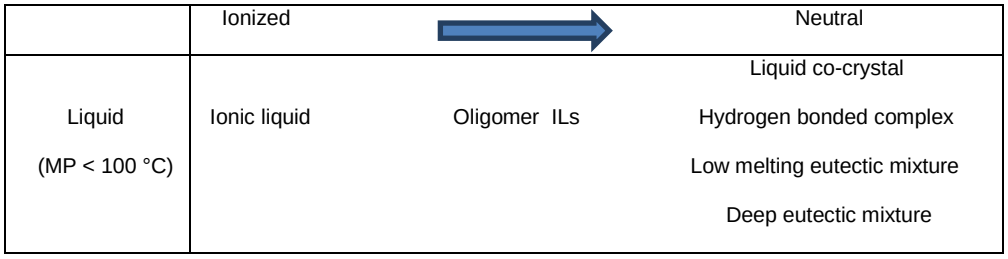


Figure 2.4 Classification of ionic liquids (Balk et al. 2015a)

Stoichiometric ratios of completely ionized anions and cations with melting points below 100 ˚C are called Ionic liquids. Liquid co-crystal, low melting eutectic mixture and deep eutectic mixture are the liquid equivalents of co-crystals, for example the liquid complex of lidocaine and fatty acids (Kelley et al. 2013),(Bica et al. 2011)and the complex of lidocaine and ibuprofen (Wang et al. 2014) (Figure 2.4). The hydrogen bonded complex formed between the acid and base with strong ion pairing was reported for the above case. There is no transference of proton between acid and base.

Oligomeric ionic liquids are the complexes of ionized and unionized form, which shared a delocalised proton, prepared when a salt is combined with an excess of acid or base. For example lidocainium with the counter ion being a complex of salicylic acid and salicylate (Bica and Rogers 2010)as well as salicylate when combined with lidocaine and lidocainium (Johansson et al. 2008).

B. Classification on the basis of properties

On the basis of the properties, Ionic liquid are classified into three generations (Figure 2.5).

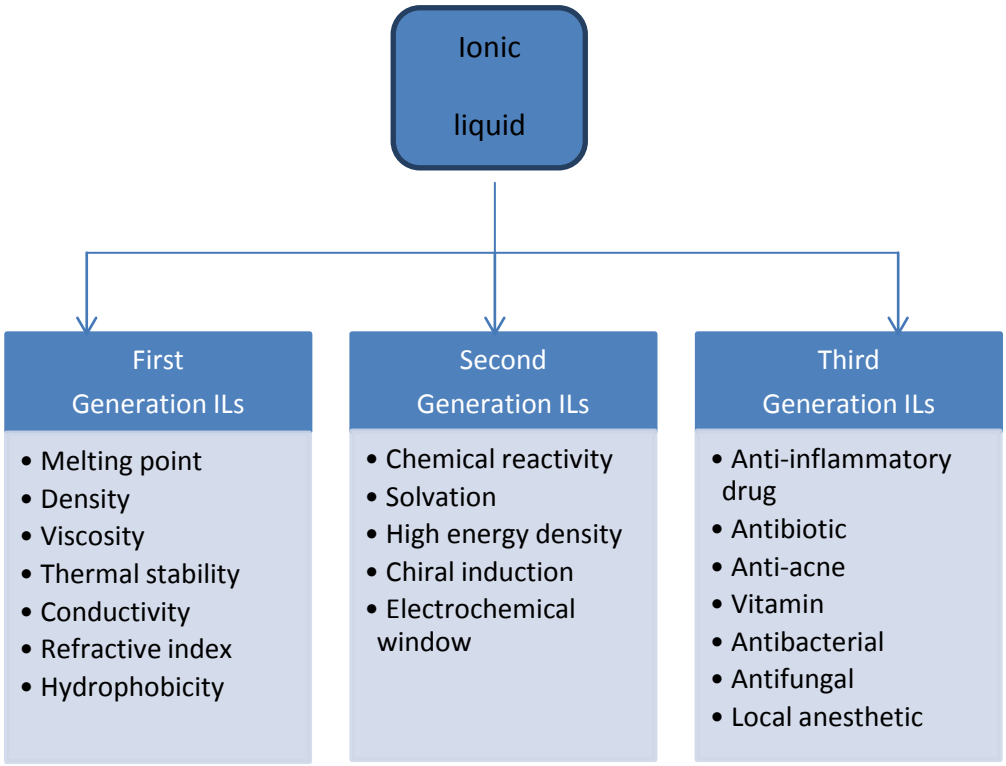


Figure 2.5 Classification of ionic liquid on basis of properties

The so-called first generation ILs were basically interesting as solvents with desirable physical and chemical properties, which includes enhanced thermal and chemical stability, non-flammable properties, negligible vapour pressure and wide liquid range as compared to molecular solvents (Freemantle 2010).

The second generation of ILs are also called task specific ILs, with desired chemical properties combined with specific physical properties. The physico-chemical properties such as reactivity, energy density, oxygen balance and many others can be tailored for specific purpose. The IL concept provides a unique architectural platform where the characteristics of both cation and anion can be modified to generate novel functional materials. For example by

combining low melting point with high thermal stability and non-volatility with desired energetic features allows an improvement in the safety concerns associated with the generation of energetic materials (Smiglak et al. 2007).

The third generation of ILs consists of those ILs which are mostly used for pharmaceutical and medical purposes such as drug delivery and drug production, formulation concept for active pharmaceutical ingredients and expanding into pharmaceutical application (Hough et al. 2007b). ILs could be designed to serve as solvents for APIs and to convert the APIs into appropriate IL salt (API-ILs). Ionic liquids were also applied as solvents for poorly water soluble drugs (Jaitely et al. 2008; Mizuuchi et al. 2008), as excipients for the preparation of microemulsions (Mizuuchi et al. 2008; Marrucho et al. 2014)and as solvents for the dissolution of APIs (Eastoe et al. 2005; Moniruzzaman et al. 2010a; Moniruzzaman et al. 2010c).

2.1.5 Toxicity, biodegradability, environment and safety of ILs

In order to utilise the potential of IL technology especially in medical and pharmaceutical applications, ILs should be devoid of toxicity and possess good biodegradability. Therefore it is significantly important to understand the toxicity of ILs along with their biodegradation, ecotoxicity, and environmental fate (Haerens et al. 2009; Busetti et al. 2010; Zhao et al. 2011).

2.1.5.1 Toxicity of ILs

The first and second generation ionic liquids have been extensively investigated in terms of their toxicity and cytotoxicity level. Ionic liquids are also called designer solvents meaning that ILs with desired properties could be easily prepared by selecting appropriate cation/anion combinations or only by changing the alkyl chain length attached to the cation. This idea led to the search of biocompatible ILs which could be generated by picking nontoxic and

biocompatible organic cations and inorganic anions (Gouveia et al. 2014).A related study based on the cytotoxicity of imidazolium, pyridinium, quinolinium and morpholinium cations illustrates that the morpholinium head group was found to be least toxic as compared with the other studied head groups (Pernak et al. 2011). ILs should be designed very carefully for medical and pharmaceutical applications as the toxicity of ILs basically depends on the cation and alkyl chain length attached to the cation. The introduction of the polar functional groups such as ether, nitrile and hydroxyl into the cation is another way to generate nontoxic ILs (Stolte et al. 2007a; Stolte et al. 2007b; Morrissey et al. 2009). Another study reported that the hydrophobic and most fluorinated anions like hexaflourophosphate and tetrafluoroborate are not appropriate for designing nontoxic and biocompatible ILs (Garcia et al. 2005). Kathrine et al reported the cytotoxicity and biocompatibility data of choline based phosphate ionic liquid. Five phosphate based anions were analysed for cytotoxicity namely: dihydrogen phosphate (DHP), dibutyl phosphate (DBP), bis(2-ethylhexyl) phosphate (BEH), bis(2,4,4-trimethylpentyl) phosphinate (TMP) and *o,o'*-diethyl dithiophosphate (DEP) using a J774 murine macrophage cell line. The EC50 is the concentration of the drug that gives half maximal response. The EC50 values of choline DHP, choline DBP and choline DEP were found to be lower than simple salts and choline chloride. On the other hand much lower values are found in case of choline TMP and choline BEH. The EC50 values of the tested ionic liquid were linked with the anion mass and the presence of moderately branched and/or long alkyl chains counterions (Weaver et al. 2010). In vitro cytotoxicity investigation was carried out using MCF7 human breast cancer cells for imidazolium, pyridinium, pyrrolidinium, piperidinium cations containing different alkyl chain lengths. IC50 is the concentration of an inhibitor

where the response is reduced to half. The IC50 values for these ILs were found to be in the range of 8μM to 44 mM. The data suggests that the presence of both the cation and anion plays a significant role in the toxicity of ILs, especially the cation with long alkyl chain length (Kumar et al. 2009).

Increased research into the biocompatibility and cytotoxicity of ILs led to the evaluation of third generation ionic liquids, which finally expanded their pharmaceutical applications. However, recent approaches for the generation of ionic liquids were attempted with those counterions which are FDA approved or are generally regarded as safe (GRAS) for example choline, artificial sweeteners such as acesulfame K, cyclamate and saccharin, quaternaryammonium counterions and p-toluenesulfate (Hough et al. 2007a; Dean et al. 2008; Dean et al. 2010b). Another very common and simple example is docusate which is combined with ranitidine, propntheline and licdocaine to give ranitidine docusate, propantheline docusate and lidocaine docusate room temperature ionic liquid (Hough et al. 2007a; Prakash 2011). Naturally occurring aminoacids and fatty acids are the other harmless option to generate room temperature ionic liquid (McCrary et al. 2013).

Hence the counterions which are generally regarded as safe or the naturally occurring molecules could be utilised to generate nontoxic, biocompatible ionic liquids. However, proper toxicological investigation should be carried out for new counterions.

2.1.5.2 Biodegradability of ILs

Currently, a number of studies have been reported which paid much more attention towards the synthesis of biodegradable ILs. The ILs with short alkyl side chain attached to the cation exhibit poor biodegradability and less toxic as compared to the same cation with long alkyl chain length (C > 6). It should be

noted that the biodegradable data of ILs shows conflict with the data reported for biocompatible ILs. ILs based on pyridinium cation were found to exhibit good biodegration potential as compared to the imidazolium based ILs which shows reduced biodegrability (Gathergood et al. 2004). Newmann et al. investigated the biodegradability of pyridinium based ILs on the basis of the presence of alkyl chain length attached to cation and functional groups (ester groups at 1- and 3- positions), suggested that the ILs with ester group shows good biodegradability under aerobic conditions. On the other hand ILs with long alkyl chains attached to cation were found to be poor biodegradable (Neumann et al. 2014).

Biodegradability of ILs is also affected by the presence of the anions. A related study has been reported that anions such as ethanoate and propanoate bearing shorter alkyl chain length were found to be not readily biodegradable. On the other hand the anions which contain longer side chain (C >3) such as butanoate, pentanoate, hexanoate and octanoate were found to be completely biodegradable (Harjani et al. 2009). In another study, the effect of anions including sulphate acetate and phosphate groups on biodegradation was investigated. These anions were found to be readily biodegradable. Extreme care must be taken while working with fluorinated anions such as hexafluorophosphate and tetrafluorophosphate because these anions are unstable in nature and can undergo hydrolytic degradation which results in the liberation of toxic and corrosive HF into the environment (Sowmiah et al. 2009). The ILs based on imidazolium, phosphonium, pyidinium and ammonium cations which shows good toxicity levels against microorganisms are found to be readily biodegradable (Wells and Coombe 2006).

2.1.5.3 Environment and safety

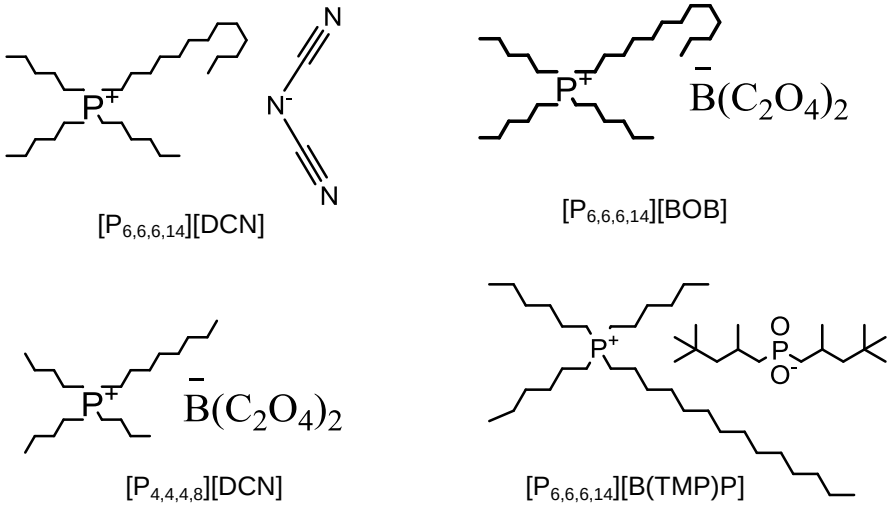
The depletion of environmental toxicity arising from chemical compounds which are used in product formulations and industrial process is one of the vital ideas of green chemistry. Sonia et al. investigated the influence of aromaticity on the toxicity of dissimilar ILs such as imidazolium, piperidinium, pyridinium, pyrolidinium containing the same anion (hexaflurophosphate). They have also worked out the impact of the position of alkyl chain length on the toxicity of IL against *vibrio fischeri, daphnia magna, pseudokirchneriella subcapitata.* The results of this study suggest that the toxicity of ILs increases with increase in the lipophilicity while the aromatic and non-aromatic ILs displays different dependencies of ionic liquid toxicity with respect to water solubility (Ventura et al. 2013). Due to negligible vapour pressure, ILs eliminates the possible risk of air pollution. A number of studies illustrate the toxic behaviour of ILs therefore the water miscibility of ionic liquid is quite necessary in order to understand the effect of ionic liquids on the environment. Mutual solubility data between imidazolium series (C_2-C_8) ionic liquid and water in the temperature range of 288.15 to 318.15 was reported. It was found that Ionic liquid solubility in water was entropically driven and the hydrophobic character of ILs increases with increase in the alkyl chain length attached to the cation (Freire et al. 2008). Although ILs are considered as green solvents, the safety aspects of ILs must be considered, especially in the synthesis of chemicals. Compatibility of ILs with reaction components need to checked before performing any chemical synthesis (Chowdhury et al. 2007). A related study suggests that the ILs with fluorinated anions such as hexaflurophosphate and tetrafluoroborate would lead to generation of hydrogen fluoride gas in the presence of water. Therefore care

should be taken while working with such types of materials and all degradation products need to be assessed (Huddleston et al. 2001).

2.1.6 Potential application of ionic liquids in pharmaceutical applications

2.1.6.1 Ionic liquids as crystallisation media and pharmaceutical solvents

The tailored properties of ILs could be explored to generate interesting discoveries. Manish Kumar et al. reported a new crystal form of nicotinamide-oxalic acid salt (2:1) by utilising the potential of ILs in crystal engineering. The phosphonium based ILs namely trihexyl(tetradecyl)phosphonium bis(oxalate)borate [P6,6,6,14][BOB], trihexyl(tetradecyl)phosphonium bis(mandelato)borate [P6,6,6,14][BMB], trihexyl(tetradecyl)phosphonium bis(2,4,4-(trimethylpentyl))phosphinate [P6,6,6,14][B(TMP)P], trihexyl(tetradecyl)phosphonium dicynamide [P6,6,6,14][DCA], 1-ethyl-3-methylimidazolium bis(mendelato)borate [EMIM] [DCA], tributyl(octyl)phosphonium bis(oxalate)borate [P4,4,4,8][BOB] are used to investigate the formation of crystal structure of nicotinamide-oxalic acid salt system (Figure 2.6). Single crystal XRD was used to analyse the structure of the synthesised salt. The nicotinamide-oxalic salt 1:1 was found to have perpendicular “tape like” structure while the other one salt with 2:1 ratio revealed two dimensional layered structure. It was also illlustrated that 2:1 salt form could only crystallised from the ILs possessing hydrogen bond acceptor functionality (Shimpi et al. 2017).



[$P_{6,6,6,14}$][DCN] [$P_{6,6,6,14}$][BOB]

[$P_{4,4,4,8}$][DCN] [$P_{6,6,6,14}$][B(TMP)P]

Figure 2.6 ILs Structures used in study (An and Kim 2012)

Another related study reported the antisolvent crystallisation technique to analyse the polymorphic design of adefovir dipivoxil. A new form of adefovir dipivoxil was generated at a temperature below 50 ºC by using 1-allyl-3-ethylimidazolium tetrafluoroborate as solvent and 1-butyl-2,3dimethylimidazolium tetrafluoroborate as antisolvent. The unique intermolecular interactions between adefovir dipivoxi and ILs allow generation of new polymorph which could not be attained by any conventional solvents. DSC and XRD tools were used to analyse the crystal (An and Kim 2012). The shape of the components of ILs used for crystallisation also plays a significant role. Generally, ILs consists of unsymmetrical and bulky ions which lead to low lattice energies due to poor packing of ions resulting in the low melting point of these designer solvents. A very distinctive environment could be generated for the crystallisation of solutes by appropriate selection of ions, where the ions can provide specific intermolecular or interionic interactions. The very first step for utilising the potential of ILs in crystallisation is the accurate selection of IL components (hydrophobic, hydrophilic coordinating/non-coordinating, hydrogen

bond donor/acceptor, which is found to be a critical (Reichert et al. 2006). Imidazolium based ILs such as 1-butyl-3-methylimidazolium hexafluorophosphate and 1-hexyl-3-methylimidazolium hexafluorophosphate were investigated for crystallisation studies. The solubility data was reported for ibuprofen and paracetamol in these ILs at a temperature range of 298.15 K – 338.15 K. It was suggested that both ILs could be utilised as solvents for pharmaceutical application (Smith et al. 2011). Studies have also been reported which aim to look towards the alternative solvents for pharmaceutical applications. Reliable solubility data was generated for isoniazid (tuberculosis antibiotic drug) using a series of imidazolium based ILs coupled with bis(trifluromethylsulfonyl)amide and trifluromethanesulfonate as anion. The study illustrated that the studied ILs (solvents) would be a good choice for manufacturing of drugs and appropriate for pharmaceutical processes. The long alkyl chain containing imidazolium IL (1-decyl-3-methylimidazolium trifluoromethanesulfonate) was considered to be the best solvent for drug among all of the ILs studied (Forte et al. 2012). A very similar study (Melo et al. 2013) was conducted with the same drug isoniazid and pyrazinecarboxamide using ammonium based ionic liquids. Isoniazid drug displays higher solubility in the studied ILs as compared to pyrazinecarboxamide drug. The ILs and drugs were analysed for their morphological properties such as phase transition temperatures, melting point, and enthalpy of fusion. Didecyldimethylammonium nitrate was considered to be the best solvent for the drugs solubility among the studies ILs. Studies have been reported which show that crystallisation can be induced due to slight exposure to water. For example procainium acetate is an ionic liquid at room temperature but extreme care should be taken while isolating it. However the presence of water gives crystalline procainium acetate

dihydrate which could not be dehydrated without decomposition. This leads to alternation of solubility and bioavailability of the active pharmaceutical ingredients. Therefore extreme care should be taken while studying crystallisation behaviour of active pharmaceutical ingredients ionic liquids (Cojocaru et al. 2013).

Studies have reported on the potential application of ionic liquids when designing polymorphs of the API (adefovir dipivoxil) in drowning-out crystallization. Due to the influence of 1-allyl-3-ethylimidazolium tetrafluoroborate on the formation of intermolecular interaction of adefovir dipivoxil in the solution, new anhydrous (N-II) and hemihydrates (N-I) polymorphic crystals of adefovir dipivoxil were reported when varying the ionic liquid fraction and crystallization temperature. The ionic liquid solvent was found to be responsible for formation of the polymorphic crystals of adefovir dipivoxil. Optimisation of ionic liquid proportion and crystallization temperature can be achieved. It is seen that there is significant enhancement in the thermal stability of adefovir dipivoxil molecules in the 1-allyl-3-ethylimidazolium tetrafluoroborate–water mixtures, due to lack of hydrolysis of adefovir dipivoxil molecules up to a temperature of 90°C. Differential scanning calorimetry studies suggest that N-I and N-II crystals were transformed into other crystal phases in the solid state (An et al. 2010). Ethambutol dihydrochloride salt exists in three polymorphic forms. Ethambutoldibenzoate salt was obtained by screening *S, S*-ethambutol free base with organic acids such as benzoic acid. Three different polymorphic forms are generated via crystallisation of ethambutol dibenzoate salt via different conditions, which are characterised by various techniques such as DSC, PXRD,IR and Raman and solid state ^{15}N NMR. Ethambutol dibenzoate salt form 2 is the least stable compared with form 1 and form 3 was observed to

be the most stable at 90°C. On the other hand at 25 °C form 1was the most stable followed by form 3 and form 2 was found to be most stable (Cherukuvada and Nangia 2012).

A polymorph can be defined as a solid crystalline phase of given compound which arises due to at least two different internal arrangements of the molecules of that compound in solid state. Different crystalline polymorphs and solvates possess different molecular conformation and/or crystal packing, as well as different crystal lattice energy and entropy which lead to differences in their physicochemical properties such as solubility, dissolution rate, melting point, density, tabletability, enthalpy, stability and bioavailability. One of the most significant benefits of room temperature ionic liquids (RT-ILs) is the ability to convert solid active pharmaceutical ingredients (API) in liquid state; the use of the liquid form of drug would eliminate the problem of polymorphism associated with solids (Song and Sohn 2011; Marrucho et al. 2014). Ranitidine hydrochloride is H2 receptor antagonist which exists in at least two different forms. Attempts to solve the polymorphism in ranitidine hydrochloride yielded to form [ranitidine] [docusate] as room temperature ionic liquid formed by using the counterion docusate (Agatonovic-Kustrin et al. 2001). A similar approach to addressing the problem of polymorphism was also attempted for propantheline bromide which is a muscarine acetylcholine receptor antagonist. This active pharmaceutical ingredient (API) was converted into room temperature ionic liquid (RTILs) by using the counterions p-toluene sulfonate and acesulfamate (Dean et al. 2008; Stoimenovski et al. 2010a). Free naproxen acid was also transferred into stable tetrabutylphosphonium based ionic liquids (TBP-ILs) with lower melting points and glass temperatures as well as enhanced solubility's and dissolution rates than the corresponding free acid (Bica et al. 2012; Balk et

al. 2015b). Other efforts have also been made to eliminate the problem of polymorphism associated with ibuprofen sodium by converting it into imidazolium based ionic liquid (Tourné-Péteilh et al. 2011).

The solubility's of drugs like paracetamol and ibuprofen were reported in 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM][PF_6] and 1-hexyl-3-methylimidazoliumhexafluorophosphate [HMIM][PF_6] at temperatures of 298.15 K, 308.15 K, 318.15 K, 328.15 K, and 338.15 K (Smith et al. 2011). In addition, solubility data for paracetamol and ibuprofen in water was reported at the same temperatures extending the data commonly reported in pharmaceutical reference texts. Solubility increment can be observed with increase in temperature. It is reported that a solvent is sufficient for pharmaceutical processing when the solubility exceeds 1 mg/ml.

The solvency of alkyl imidazolium salts (PF_6, Br, Cl) for poorly water soluble model drugs, albendazole and danazol, indicating their potential application as pharmaceutical solvents/cosolvents has been reported by Mizuuchi et al. The solubility of albendazole was found to increase by more than 10,000 times in 1-butyl-3-methylimidazolium hexaflourophosphate [BMIM][PF_6] (Mizuuchi et al. 2008). The aqueous miscibility of a poorly water soluble RTIL such as [BMIM][PF_6] can be improved by inclusion of a second more miscible RTIL (e.g. 1-hexyl-3-methylimidazolium bromide [HMIM][Br]. Furthermore the extent of improvement in water miscibility was found to correlate with the hydrophilicity of the second RTIL. This ability to modulate the water solubility of RTILs increases their usefulness as pharmaceutical solvents. In addition, studies suggest negligible toxicity of [BMIM][PF_6] and [HMIM][PF_6] towards caco-2 cells, though a slightly higher toxicity is observed in the same test system with the [OMIM][PF_6] salt, which is surface active (Figure 2.7).

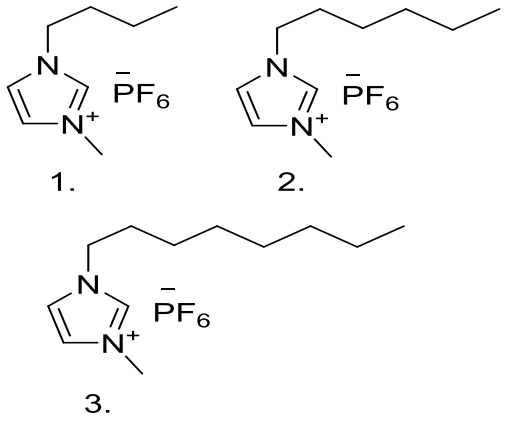


Figure 2.7 Structures of [BMIM][PF6], [HMIM][PF6], [OMIM][PF6]

2.1.6.2 IL as carrier for drug delivery

The successful application of novel IL-assisted non-aqueous microemulsion stabilized by a blend of two nontoxic surfactants, polyoxyethylene sorbitan monooleate and sorbitan laurate for transdermal delivery of acyclovir, which is insoluble or sparingly soluble in water and most common organic liquids was demonstrated (Moniruzzaman et al. 2010b).

The influence of ILs on emulsion properties was studied by incorporating ILs into the emulsion structure, resulting in stable formulations. The conventional oil-in-water (O/W) and water in oil (W/O) emulsions containing ionic liquids such as the hydrophilic IL [HMIM][Cl] and hydrophobic IL [BMIM][PF_6] were prepared resulting in stable formulations. The antimicrobial activity of ILs in the formulations was analysed and their application as preservatives was confirmed by performing preservative efficacy tests. Evaluation of the in vitro cytotoxicity of the emulsions containing hydrophilic or hydrophobic ILs showed the low cytotoxicity of the carriers. Finally penetration enhancement of a fluorescent dye (Fluorescein sodium) as a model drug in the presence of ionic liquids was shown (Dobler et al. 2013).

The influence of imidazolium ionic liquids on bio-chemical parameters that influenced the *in vivo* behaviour of nimesulide was studied. In this context, the binding of nimesulide to human serum albumin (HSA), in IL media, was studied. In addition to that the evaluation of the interaction of drug-IL systems, with micelles of hexadecylphosphocholine (HDPC), enabled the calculation of partition coefficients (k_p). Both assays were performed in buffered media in the absence and in the presence of [EMIM][BF_4], [EMIM] [Ms] and [EMIM] [TfMs]. Even though there was an increase of the dissociation constant (k_d) in IL media, nimesulide still binds to HSA by means of strong interactions. The thermodynamic analysis indicated that the interaction was spontaneous for all the tested systems. Moreover, the studied systems exhibited properties that were favourable to the interaction of the drug with biological membranes, with (k_p) values 2.5-3.5 higher than in aqueous environment. The studied nimesulide-IL systems presented promising characteristics regarding the absorption and distribution of the drug in vivo, so that studied solvents seem to be good options for drug delivery (Azevedo et al. 2013).

Studies have been carried out on a novel ionic liquid-in–oil microemulsion capable of dissolving drugs which are poorly water soluble or insoluble in water and most of the pharmaceutically accepted organic liquids. This work comprised the formation of nanometer-sized ionic liquid droplets in isopropyl myristate with a blend of nonionic surfactants like polyoxyethylene sorbitan monooleate and sorbitan laurate. Results showed that the ionic liquids containing strong hydrogen bond acceptor coordinating anions played a significant part in the formation of microemulsion droplets, when a set of ionic liquids was tested as dispersed phase. The shape, size and size distribution of the aggregates in the characterized microemulsions illustrates the formation of spherical micelles in

the range of 8-34nm. The study was extended to the solubility of some insoluble or sparingly soluble drugs such as acyclovir, methotrexate, and 1-[(5-(p-nitrophenyl) furfurylidene) amino] hydantoin sodium) and a high degree of solubilization in the IL microemulsions observed. Thus the unique physical, chemical and biological properties of microemulsions formed with ILs offer novel opportunities to develop a potential drug delivery carrier for poorly soluble drug molecules (Moniruzzaman et al. 2010a).

Hydrophobic RTILs can be useful as versatile solvents and matrices for designing of controlled release drug delivery systems. Some relevant properties of [BMIM][PF_6], [HMIM][PF_6], [OMIM][PF_6] were studied and explored, indicating that this set of ionic liquids exhibited low aqueous solubility with increasing alkyl chain length and could be easily understood by the given data as : 0.035 mol/l, (bmim), 0.032 mol/l, (hmim) and 0.09 mol/ l (omim). The release of sucrose and dexametasone from water immisible RTILs deposited into water was prolonged over 48 hours. The saturated solution of these RTILs demonstrated little toxicity towards caco-2 cells, although the (omim) derivative, which is more surface active, has a small effect on cell viability. Water immiscible ionic liquids are interesting reservoirs for electrically controlled release of insoluble or sparingly soluble drug molecule. An enhancement in the drug release profile of some solutes in water is observed on passage of electric current through these ionic liquids (Jaitely et al. 2008).

The influence of the 1-butyl-3-methylimidazolium tetrafluoroborate on drug-surfactant association as binder has also been explored. The association between the drug curcumin and anionic surfactant sodium dodecyl sulfate was found to be increased in presence of IL. On addition of the IL, the positive charged head groups of the imidazolium cation help to stabilize the negative

charges of the sulfate ion of anionic surfactant sodium dodecyl sulfate and the deprotonated curcumin. On the other hand, repulsion was observed between the deprotonated form of curcumin and negative head groups of sulfate ion. The presence of the IL dramatically increased the association of curcumin and neutral Triton X-100 (TX100), this could be attributed to the hydrogen bonding and the dipole-dipole force of attraction between the positive charge of the head group of the Il and TX-100. This leads to strong interactions and associations of curcumin with the neutral surfactant solutions (Patra and Barakat 2011) (Behera et al. 2007).

Another similar study investigates the binding ability of ionic liquid (1-tetradecyl-3-methylimidazolium bromide) for the acetylcholine chloride and dopamine hydrochloride. The results for the binding ability of the IL was compared to the conventional cationic surfactant tetradecyltrimethylammonium bromide [TTA][Br]. The IL (1-tetradecyl-3-methylimidazolium bromide) was found to possess good binding ability and an efficient drug carrier for both drugs. Both surfactant molecules act as good binder for dopamine hydrochloride as compared to acetylcholine chloride due to cation-π interactions between the positive charge of the surfactant molecules and aromatic region of the dopamine hydrochloride. Among both surfactant molecules the IL was found to have good binding ability to the dopamine hydrochloride due to π-π interactions between the aromatic region of the dopamine hydrochloride and the imidazolium ring of the IL (1-tetradecyl-3-methylimidazolium bromide). The polar head groups repulsion in the ionic liquid (1-tetradecyl-3-methylimidazolium bromide) was found to be greater than [TTA][Br] as the positive charge in the imidazolium ring is delocalized, which leads to create steric hindrance and finally helping to form aggregates (Figure 2.8). The polar head group of the IL

allows having lower critical micelle concentration (CMC) as compared to [TTA][Br] (Mahajan et al. 2012).

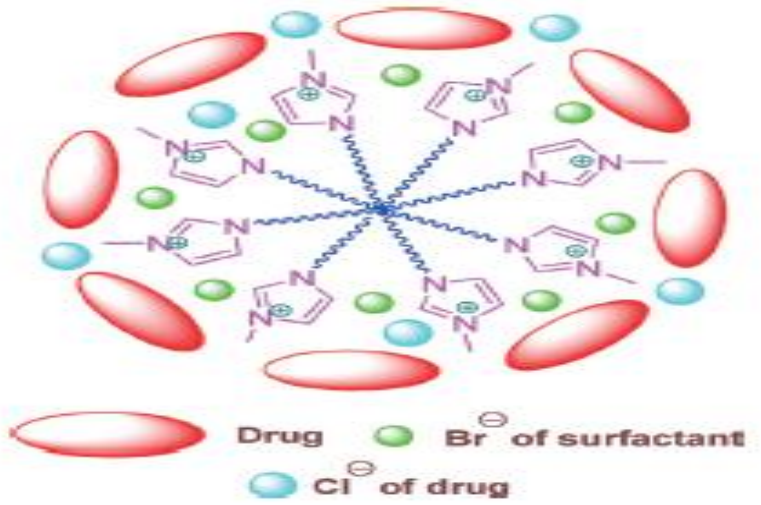


Figure 2.8 Possible interaction between drug and IL (Mahajan et al. 2012)

Williams and colleagues have exploited the potential of ionic liquid in oral drug delivery systems by using a poorly water soluble drug danazol. In this study self-emulsifying drug delivery systems (formulations) were made by incorporating ILs. Then danazol was loaded into these formulations and has been evaluated for its dissolution profile in simulated gastric and intestinal fluids after dispersion. IL based formulation containing danazol was found to give 4.3 fold higher exposure than crystalline drug and lipid formulation. *In-vivo* results shows that IL based SEDDS were found to be highly effective in keeping danazol in a solubilised state. IL-based SEDDS formulations allow high drug loading ability, facile dispersion in GI fluids and insensitivity to GI digestive systems (Williams et al. 2014).

2.1.6.3 Drug ionic liquid

The significant aspect of drug development is the design and synthesis of pharmaceutically acceptable salts. Almost more than half of all drugs used are administered as salts. The important pre-formulation task is the salt formation of the desired drug molecule as it improves the water solubility, industrial processing, safety aspects and sometimes biological properties. The replacement of the counter-ion of the active moiety can enhance these characteristics. Collating all these gives an idea of utilizing ionic liquids in the pharmaceutical industry that is fascinating and inspiring. A number of ionic liquid formulations with known therapeutics have been reported. Any combination of two or more drugs that have appropriate and opposite ionic forms are possible as long as both active ingredients form a stable cation and anion. In this way the newly formed drug comprises an IL containing an API moiety which may be either cation or anion and a counter-ion component which is selected from the generally regarded as safe (GRAS) list is potentially a non-toxic, pharmaceutically acceptable compound. The formation of drug ionic liquids offers novel and unique properties which may not be expressed by the standard crystalline salt forms of the API currently available in the market. Newly formed drug ionic liquids render enhancement in the dissolution profile of the drug molecule which can be utilized through different administration by selection of a counter-ion that is appropriate for tailoring the physical and chemical properties to a chosen administration route (Ressmann et al. 2011) .

Currently, half of all the API molecules used are administered in the form of salts; the preparation and formulation of suitable salt as drug candidate are considered as vital steps in the preclinical phase of modern drug discovery. A

number of reports could be found in the literature suggesting the formation of salt just by pairing active pharmaceutical ingredients (API) in the cation or anion form with biocompatible or inert counterions (Sekhon 2011).

Ranitidine hydrochloride exists in two different crystalline forms. Room temperature ionic liquid (RTIL), [ranitidine][docusate] was made to overcome the problem of polymorphism by combining ranitidine and the counterion docusate (Hough et al. 2007a). Liquid aspirin is synthesised by ion-exchange reaction between acetyl salicylate and pharmaceutically acceptable ammonium salt. The author's report significant enhancement of the pharmacological properties such as bitter taste, poor solubility, and large tablet required for the dosage of the prepared salt over solid aspirin (Bica et al. 2010). Another similar study was reported in which salicylic acid (SA) was used as a model drug, combined with imidazolium cation with varying alkyl side chains to generate a room temperature ionic liquid. Three different types of API-ILs were prepared: IL bearing SA as anion, IL bearing SA as cation or anion, IL bearing SA as cation. The cytotoxicity studies were carried against human colon cancer cell line and were found to be significantly higher than the pure SA and that of conventional imidazolium based ILs. The synthesised API-ILs showed higher water solubility when compared to SA (Egorova et al. 2015).

Cole et al, synthesised the antibiotic ampicillin based ILs by employing metathesis reaction between cationic pyridinium, imidazolium and sodium salt of ampicillin. The objective of this study is to conquer antibiotic resistance, particularly in β-lactam drugs. The antibacterial activity of the synthesised ampicillin based ILs and their parent materials were studied and the results suggest that the synthesised ILs exhibited enhanced antibacterial activity compared to their parent materials. Thus this study illustrates the feasible

alternative as antibacterial agents (Cole et al. 2011b). Another similar study used a different method for synthesising ampicillin based ILs. The author first made basic ammonia solution of ampicillin which was then neutralised with the different organic cation hydroxides namely: [EMIM], [EOHMIM], [Chol], [TEA] and [$P_{6,6,6,14}$]. The salts prepared by this method exhibited greater thermal stability and water solubility compared to parent starting material (Ferraz et al. 2012). Later in a subsequent study, ampicillin based IL was explored for their bacterial and growth inhibition properties against a number of sensitive bacteria, most specifically Gram-negative resistant bacteria. The ampicillin mechanism of action was significantly influenced by ion-pair combination, hence the authors selected hydrophobic ampicillin moiety for this study. Among all ILs studied, [C16Pyr][Amp] displays highest inhibition. Although the rest of the ILs also gain better results compared to the control [Na][Amp] and starting chloride and bromide salts (Ferraz et al. 2014b). The pharmacological properties such as partition coefficient (octanol-water), water solubility, and critical micelle concentration of ampicillin based ILs were also studied. Among all the studied ILs, [Chol] [amp] was the IL found to exhibit enhanced water solubility and octanol-water partition coefficient compared to starting materials (Florindo et al. 2013).

Another study (Pinto et al. 2013) was conducted, where salicylate based IL such as [cetylpyridinium][salicylate], [benzalkonium][salicylate] and [EMIM][salicylate] were explored for their pharmacological properties such as partition coefficient, critical micelle formation and protein binding ability. The results suggest that all the studied ILs displays the ability to form micelles and bind strongly to human serum albumin. The partition coefficient values were found to be 6 times greater than that of starting materials. Interestingly, the IL

salts display more affinity for the lipid phase compared with the starting materials.

Ionic liquids prepared with cholinium cation are biocompatible, which is essential nutrient for the normal growth of cells and its deficiency in diet caused fatty liver and some other abnormalities in humans and adult animals. Keeping this in mind, a study of novel cholinium based API-IL was accomplished from nalidixic acid, niflumic acid, 4-amino-salicylic acid, pyrazinoic acid and picolinic acid. The API-IL was prepared by a simple, two step anion exchange reaction. The parent materials along with the synthesised API-ILs were subjected for their solubility studies to both water and buffer solutions at 25 °C and 37 °C. The biopharmaceutical properties especially water solubility, melting point, bioavailability and membrane permeating ability were found to be enhanced compared with the parent active pharmaceutical ingredients. *In-vitro* cytotoxicity studies have been conducted to explore the cytotoxicity level through two different human cell lines, Caco-2 colon carcinoma cells and HepG2 hepatocellular carcinoma cells for both the synthesised cholinium based API-ILs and the starting materials. The most interesting results were obtained for [chol][nalidixate] and [chol][niflumate], whose starting APIs were nearly water insoluble(solubility < 0.1 mg ml^{-1}) (Araújo et al. 2014).

Another similar study reports the potential of transforming poorly soluble acidic active pharmaceutical ingredients into tetrabutylphosphonium salts. Metathesis reaction was carried out to generate tetrabutylphosphonoium based API-ILs from the sodium salts of the poorly water soluble acidic APIs (Diclofenac, Ibuprofen, ketoprofen, naproxen, Sulfadiazine, Sulfamethoxazole, Tolbutamide). The melting point and glass transition temperature of tetrabutylphosphonium salts were found to be lower compared with the starting material and the

solubility profiles of the synthesised salts were enhanced to be 1000 times greater than the parent APIs (See Fig. 2.9). The increase in the dissolution profiles of the tetrabutylphosphonium salts was expected due to the increase in hygroscopicity. Solubility issues associated with the poorly water soluble APIs could be addressed via API-IL concept (Balk et al. 2015).

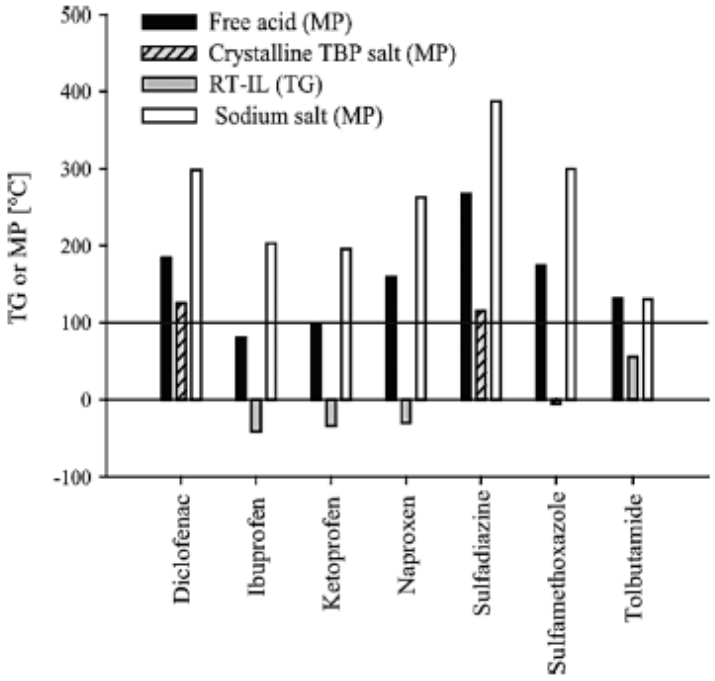


Figure 2.9 The glass transition temperatures and melting points of APIs, sodium salts and tetrabutylphosphonium salts (Balk et al. 2015)

Alves et al. reported that the model drug tetracycline when combined with the biocompatible anion docusate allows tuning of hydrophilicity of salts. Some of the important properties for antibiotics such as hydrophilic-hydrophobic balance and water solubility were studied. The API-IL tetracycline docusate, was studied and characterised in terms of water solubility, thermal stability and UV-vis spectrophotometry was utilised to study octanol-water and liposome-water partition coefficient as tetracycline display absorbance around 270nm. The interactions of newly synthesised API-IL (tetracycline docusate) and tetracycline

hydrochloride with liposomes were analysed by determination of partition coefficient using derivative spectroscopy. Egg yolk phosphatidylcholine liposomes were utilised as model for cell membrane. The octanol-water and liposomes-water partition coefficient data suggest favourable partition towards lipophilic phase. The water solubility of tetracycline-docusate was decreased to half when compared to parent drug tetracycline hydrochloride (Alves et al. 2013).

Recently the API-IL strategy was applied to form ketoconazole citric acid and ketoconazole tartaric acid ionic liquids. Ketoconazole is synthetic antifungal drug containing imidazole ring which is insoluble in water, while citric acid and tartaric acid are pharmaceutical acceptable counter-ions. Solvent evaporation technique was employed to synthesise ketoconazole citric acid and ketoconazole tartaric acid ionic liquids using different molar ratios. The results of this study suggest that the solubility of the synthesised ILs increase with increase in the molar ratio of the citric acid and tartaric acid. Only slight difference of solubility was observed between the physical mixture and non-processed ketoconazole. The findings of this study offer number of attractive advantages such as increased in solubility, overcome polymorphism issue and disadvantages of ketoconazole (Keramatnia et al. 2016).

The use of silica-supported IL phase (SILP) to immobilise API-ILs on mesoporous silica has been demonstrated by using stability, sorption and release properties of two ILs namely, tetrabutylphosphonium ibuprofenate [P 4444][Ibu] and lidocainium ibuprofenate [Lid][Ibu]. This is a simple technology used to handle and dose liquid formulations. One of the attractive features of silica supported ionic liquid (SILP) delivery systems is the ability to fine tune and to control the adsorbed IL release by altering the design of ionic liquids. This

technology could also act as a device for drug delivery with complete and rapid release of the drug from the silica carrier when place in aqueous environment (Bica et al. 2012).The degree of ionicity has been evaluated for 28 PILs using NMR spectroscopy. It is well known (Moreira et al. 2015) that the ionic drugs do not permeate easily through biological membrane. The aim of the study was to understand the effect of ionic state in the API combinations and their solutions in DMSO. A series of acids such as salicylic acid, flurbiprofen, flufenamic acid, diclofenac, two artificial sweeteners (saccharin and acesulfame K, docusate (a laxative) with three bases: lidocaine, prilocaine, bupivacaine and an antibiotic drug metformin combines to generate the desired protic ionic liquids. This study provides an easy and accessible procedure to analyse the degree of ionicity of protic ionic liquids based on NMR spectroscopy (Moreira et al. 2015).

Similar study was reported which focused on the issue of proton transfer and salt formation in the family of pharmaceutically active acids and bases. The objective of the study was to understand the factors responsible to control H-bonding, melting point and proton transfer and accordingly the authors selected a set of acids (benzoic acid, salicylic acid, gentistic acid) and bases (Amantadine, tuaminoheptane, 2-Pyrrolidinoethanol)with minor change in structure and in physicochemical properties. The properties of the salts are governed by the degree of ionocity (protonic character). The proton transfer and the extent of ion association in these salts were studied by FTIR-ATR and transport properties (Waldon plot). The results show that strong ionic interactions were observed in primary amine due to the formation of extended hydrogen bonded clusters in salts which is the primary dominating factor for membrane transport properties of these compounds *in vivo* studies (Stoimenovski et al. 2012). Studies have been reported to overcome the

problem of hygroscopicity of antitubercular drug ethambutol. A number of protic acids (adipic acid, fumaric acid, p-toluenesulfonic acid, benzenesulfonic acid, methanesulfonic acid) were used to obtain salt form of the drug. These salts were characterised by ATR-IR, NMR, TGA, DSC and XRD. The results showed that the new products were also found to be hygroscopic (Cherukuvada and Nangia 2013).

Tourné-Péteilh et al. (2014) have been reported to understand the interactions between imidazolium cation and ibuprofenate anion in a set of imidazolium based ionic liquids varying alkyl chain length (n = 4, 6, 8). The authors also interestingly studied the structure and composition of the aggregates which form on self-assembling in water. The techniques used to understand the interactions between the counterions are dynamic light scattering, cryogenic transmission electron, ^{1}H NMR spectroscopy, and atom-scale molecular dynamics simulations. Surface tension and conductivity measurements were used to determine the CACs (critical aggregates concentrations) of the imidazolium based ibuprofenate ionic liquids and were found to be lower for ILs compared to parent materials. Mixed micelles were produced at high concentration and the composition basically depends upon the alkyl chain length of the imidazolium cation. Stoichiometric composition aggregates were formed upon increase in the number of alkyl chain length and composition became enriched in imidazolium cations. Micelles were mainly composed of ibuprofenate anion with some of the imidazolium cation, when the chain length is very short. The change in the chemical composition and the dilution effect are commonly responsible for the transitions from micelles to vesicles or ribbons. Thus it can be concluded that the ibuprofenate imidazolium cation association could be

considered as an ion-pair amphiphile displaying very low interfacial activities (Figure 2.10) (Tourné-Péteilh et al. 2014).

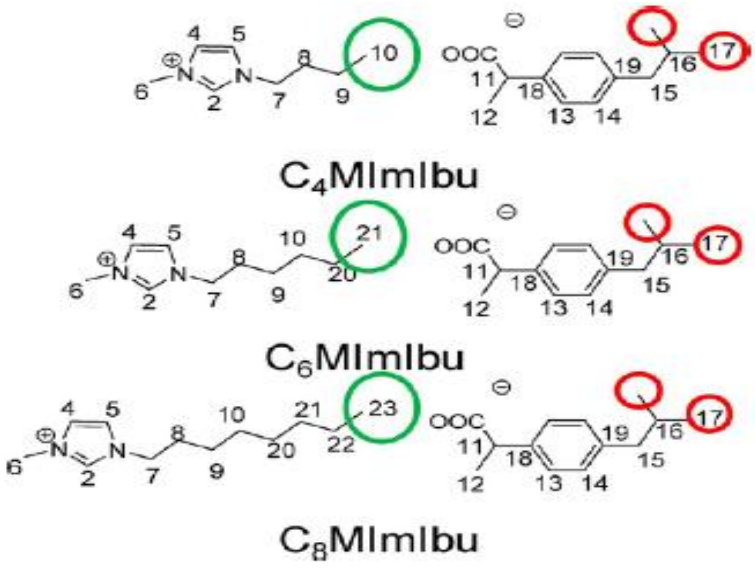


Figure 2.10 Chemical structure of investigated imidazolium based ibuprofenate ILs

The red and green circles indicate the tail groups of the anion and cation (Tourné-Péteilh et al. 2014). The IL concepts of combining the biological property of quaternary ammonium compounds (QACs) with the biological activity of non-nutritive sweeteners such as saccharinate and acesulfame have been explored for pharmaceutical applications. Eight ILs namely: didecyldimethylammonium saccharinate, didecyldimethylammonium acesulfame, benzalkonium saccharinate, benzalkonium acesulfame, hexadecylpyridinium saccharinate, hexadecylpyridinium acesulfame, 3-hydroxy-1-octyloxymethylpyridinium saccharinate and hydroxy-1-octyloxymethylpyridinium acesulfame have been synthesised and characterised. The anti-microbial study of the six ILs have been studied, which shows no trend some of the ILs displays decreased anti-microbial activity while few exhibited significant increase in anti-microbial activity. Oral toxicity,

deterrent activity and skin irritation of didecyldimethylammonium saccharinate, didecyldimethylammonium acesulfame have been conducted and the results shows both of the ILs achieved category 4 (harmful) for oral toxicity and skin irritation, while the deterrent activity of both ILs was found to be good or very good against various types of insects (Hough-Troutman et al. 2009).

(Balk et al. 2015a) reported to prodrug notion to compare with IL approach, a poorly soluble drug α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonist was converted into IL in order to look for faster and higher oral availability compared to prodrug. The structural confirmations data was collected via NMR, DSC, IR, liquid state NMR and ESI-MS. The impact of counterion on solubility profiles, dissolution and precipitation kinetics were evaluated. Cytotoxicity studies were carried out in three cell lines of hepatic and renal origin in macrophages. Results shows that the IL strategy has profound influence on the physicochemical properties of API properties without any structural changes in the actual drug, which is required in prodrug approach. The dissolution profile of IL was found to be 700 fold faster than free acid, transepithelial transport was three times faster for IL compared to prodrug and the counterion was found to be nontoxic with IC50 (Balk et al. 2015a).

Some of the challenges faced by the pharmaceutical industry can be resolved by the use of ionic liquids as RTILs offers advanced and original solutions in new treatment and drug delivery systems. For example researchers can synthesize an API in liquid form as liquid salt, which will not be receptive to polymorphism. This can be achieved by pairing an API of known characteristics with a counter-ion of notable tendencies resulting in an ionic liquid having a low melting point. It also seems that negative side effects of given active drug moiety can be prevented by delivering it as ionic liquid where the oppositely

charged active compounds nullify the side effects. Dual treatment therapies can be achieved by combining two active ions. For example, the local anaesthetic lidocaine was proved to have enhanced and prolonged effect on rats when applied as an ionic liquid in the form of lidocainium docusate when compared to the commonly used solid hydrochloride salt (Rodríguez et al. 2009).

Bica et al. (2010) prepared novel ionic liquids by using simple ion exchange reactions between salts of the active component of aspirin, or chemically similar salicylic acid and pharmaceutically active ammonium salts. Later on the counterion could be used to add a second function to the ionic liquid drug such as antibacterial or antimicrobial behaviour. The data suggest that the limited stability of acetylsalicylate ILs prevents pharmaceutical application(Bica et al. 2010). Many potential drugs fail to perform a successful action as they do not fulfil their potential due to poor solubility. Such hurdles can be nullified by utilizing ionic liquids in drug formulations.

A recent study has been published which focuses on IL-based oral drug delivery. The objective of this work is to increase oral exposure by enhancing solubility in lipid based formulations and integration into lipid solubilisation pathways. Poorly water soluble drugs such as itraconazole, halofantrine and cinnazarine are converted into lipophilic ionic liquids by metathesis reaction. The reaction has been carried between hydrochloride salts of the drugs and the range of lipophilic counterions such as sodium oleate, sodium dodecyl sulfate, sodium docusate, sodium stearate, ammonium decylsulfate and ammonium octadecylsulfate. The API-ILs synthesised are either completely soluble or showed enhanced solubility in lipid formulations or formulations based on SEDDS. Itraconazole docusate and cinnazarine decylsulfate were completely dissolved at high concentration in SEDDS formulations to achieve high

exposure. Enhanced drug absorption was observed for itraconazole up to 20 fold and for cinnazarine up to 2-fold compared to controlled suspension formulations. This study suggests that the synthesised lipophilic ionic liquids provides an alternative pathway to enhance drug solubility profile in SEDDS formulations and therefore overcoming the challenge associated with oral drug delivery systems (Sahbaz et al. 2015).

2.1.7Skin as biological barrier

Skin is the largest organ of the body which acts as a promising gateway for systematic or local action (Figure 2.11). The main function of skin is to act as barrier between the outer environment and the body. Human skin is composed of four main regions: the stratum corneum, epidermis, dermis and hypodermis. Stratum corneum is made up of phospholipids, cholesterol sulfate, protein (75-85%), lipids (5-15%) and forms the outermost layer of the skin which acts as the actual barrier to the most of the substances. Epidermis is found between the stratum corneum and dermis having thickness range from 50-100 µm. The water content found in epidermis is about 90%. Dermis lies beneath the epidermis and having thickness range between 2000-3000 µm. It is composed of fibrous protein (Singla et al. 2012).

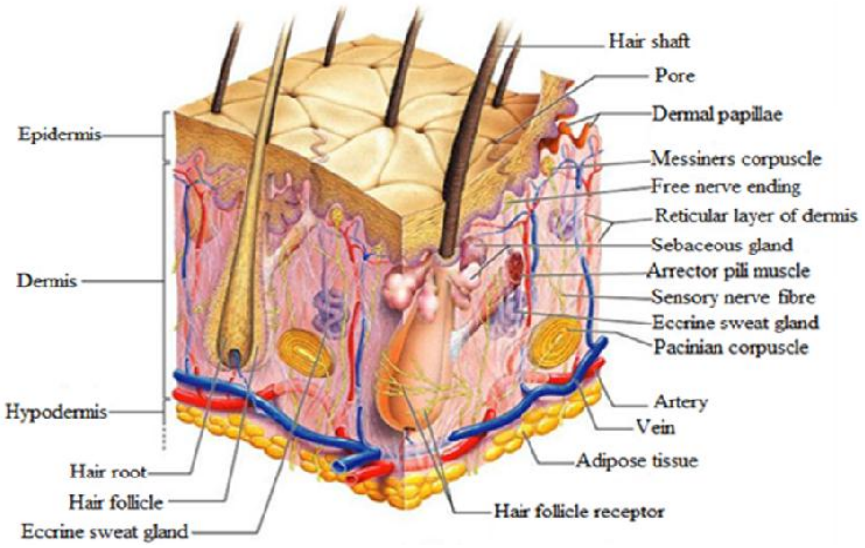


Figure 2.11 Cross section of human skin (Singla et al. 2012)

2.1.8 Drug administration to the skin

Topical and transdermal drug delivery has been attracting a huge interest throughout the globe as it offers many potential benefits over other routes such as avoiding local gastrointestinal irritation and hepatic first-pass effect and providing improved patient compliance (Ranade 1991). The topical application of drug can be used to treat diseases of the outer part of the skin or can allow reach to the deeper tissues without any at all potential side effects. The active ingredient used to treat certain local muscuskeletal inflammations and osteoarthritis requires penetration as deep as underlying muscles. The therapeutic drug levels to treat acne should reach deeper into epidermal layers in order to target pilosebaceous structures in psoriasis disease. The domain of deep tissue penetration after topical application of drug gained limited attention (Guy and Maibach 1983). The topical delivery of local anaesthetics to deeper layers of skin would be the most beneficial in case of paediatric patients and for those patients who are uncomfortable with hypodermic needles.

2.1.9 Drug penetration routes

The strictly ordered structure of stratum corneum makes the transdermal permeation of active pharmaceutical ingredients difficult or often impossible. The permeation of the drug molecules via skin basically includes diffusion through intact epidermis and through skin appendages such as sweat glands, hair follicle via shunt pathways through intact epidermis. However the surface area of these skin appendages are very low (0.1 %) of the total human skin and drug delivery via this route is considered to be very small. The drug delivery pathway is basically influenced by the formulation used and the physicochemical properties of the compound. The transcellular pathway is more favoured when penetration enhancer is used as it increases the permeability of the corneocytes by changing the keratin structure. The molecule passes through the cells in the transcellular route. The transcellular pathway is not considered under normal conditions due to very low permeability via corneocytes and due to problematic partitioning several times from hydrophilic corneocytes into lipid intercellular layers of stratum corneum and vice versa (Figure 2.12) (Kolesnikova et al. 2013).

In intercellular pathway, the molecule passes through the spaces found between the cells, although it is the longest pathway but also one of the most prominent route compared to other pathways. Intercellular route allows hydrophilic substances to pass through via hydrophilic route and lipophilic substance via lipophilic route as this route provides both regions (hydrophilic and lipophilic) (Ehrhardt and Kim 2007).

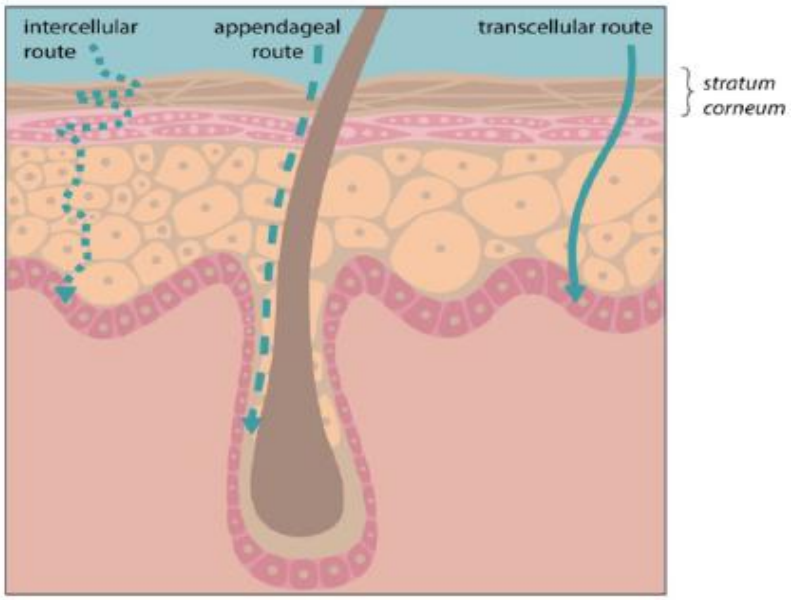


Figure 2.12 Different penetration routes via SC (Williams and Barry 2012)

The drug molecule must first dissolve in the vehicle in order to diffuse from stratum corneum/vehicle interface. Then the drug particles partitions into stratum corneum and diffuse through it and then partitions into the viable epidermis. The partitioning of lipophilic drug through viable epidermis is not favourable because of the hydrophilic nature of the epidermis which results in the deposition of lipophilic drug into stratum corneum. The drug moves into blood for systemic removal after passing into the dermis. The skin can be considered as a trilaminate structure comprising the outermost dense keratinized layer called stratum corneum, followed by viable epidermis and then the dermis.

2.1.10 Formulation and drug modification for topical/transdermal drug delivery

This section will provide brief insights into alternative chemical strategies which can be used to modify topical and transdermal drug delivery.

2.1.10.1 Ion pairing

Charged species do not readily permeate via the human skin membrane. This drawback could be overcome by formation of an ion pair which is formed by combining an oppositely charged species with the charged permeant; in theory, a complex in which the charges are neutralised allowing the permeant to penetrate through membrane where it may dissociate to eliminate the charged species. Scott et al.(2001) suggested that propranolol formed a 1:1 addition complex with many fatty acids during investigating the permeation of β-blocker via human skin, for instance lauric acid and β-blocker when applied concurrently they permeate at a rate of 1:1 mole ratio through human skin membranes (Stott et al. 2001). Studies have been reported where salicylates was paired with quaternary ammonium ions and amines (MEGWA et al. 2000). Smith and Irwin reported that ion pairing was not only responsible for increase in flux through human skin membrane by penetration enhancers (Smith and Irwin 2000).

2.1.10.2 Eutectic systems

In addition to the properties such as partition coefficient and molecular weight, the melting point of the permeant has significant impact on transdermal permeation. The lower the melting point of the drug molecule the greater its solubility in skin lipids including in a given solvent. Eutectic formation is one of the methods by which the melting point of the drug delivery system can be lowered. Eutectic binary mixtures are formed by combining two components that do not interact with each other and form a new chemical entity which at a

certain ratio inhibits crystallisation process of one other resulting into the formation of eutectic composition. EMLA cream was the first commercially successful eutectic formulation formed by mixture of lidocaine and prilocaine (mixture of local anaesthetics) (Nyqvist-Mayer et al. 1986).

The use of eutectic mixtures was gaining interest in the field of topical drug delivery. The mixture of ibuprofen with a series of terpene penetration enhancers was reported to form eutectic mixture for improving transdermal drug delivery. Among the mixtures studied terpene penetration enhancer thymol was found to form at eutectic mixture which melts at 32 °C (Stott et al. 1998).

2.1.10.3 Prodrugs

Prodrug formation was one of the earliest and most successful strategies employed for both systemic and local delivery of topically applied drugs. Generally, the prodrug formation was designed for lipophilic drug molecule because the increase in lipophilicity will help in partitioning of the molecule into stratum corneum. Some polyoxyethylene ester prodrugs of ketoprofen, naproxen and diclofenac were reported and were found to increase percutaneous absorption *in vitro*, and also provided with sustained *vivo* effect (Bonina et al. 2001).

2.1.10.4 Supersaturation

The thermodynamic activity of any formulation can be increased beyond unity by using supersaturated systems. Supersaturation in any topical formulation can be achieved by evaporation of a solvent or co-solvent. Supersaturation of the drug molecule in the donor phase results in the uncontrolled and unpredictable evaporation of the vehicle from the skin surface. Many topically applied formulations are thought to operate probably by this mechanism (Coldman et al. 1969). These systems can also be formed by cooling a warm

saturated solution back down to skin temperature resulting in increasing thermodynamic activity of the molecule (HENMI et al. 1994).

2.1.11 Summary of background

This section sheds light on the definition, history, physicochemical properties and potential pharmaceutical applications of ILs. The structure of skin, different drug penetration routes via skin, formulation and drug modification required for efficient topical and transdermal drug delivery was briefly described in background section.

2.2Literature review

As illustrated in the introduction part that this study was focussed on topical application of ILs, therefore this section gives detailed review of IL on topical and transdermal application.

2.2.1 Significance of IL in topical drug delivery systems

Different drug delivery systems have been developed with varied targeted delivery routes including topical, transdermal, oral, injection and nasal during the last two decades. The transdermal and topical drug delivery method seems to be ideal among the different routes as it is considered as a safest route for drug administration. Topical and transdermal application of drug offers several advantages over the conventional mode of drug delivery systems, as it surpasses first pass metabolism, increased patient compliance and depletion of few side effects. This method also helps to reduce the loss caused by metabolism in the oral cavity, gastrointestinal tract, liver and also offers the advantage of increase bioavailability (Herkenne et al. 2008).

A number of active pharmaceutical ingredient-ionic liquids (API-ILs) have been reported, which are the liquid form of active pharmaceutical ingredients. API-IL

approach offers several attractive advantages over crystalline pharmaceuticals such as removal of polymorphism and enhanced bioavailability (Hough and Rogers 2007). API-IL approach helps to enhance the pharmacodynamics and pharmacokinetics property of the active pharmaceutical ingredients via administration of dual drug instead of two individual salts. The degree of ionicity and the molar proportion ratio of the counterion and the API are the critical characteristics which are directly related to the pharmaceutical industry as these properties have significant influence on the bioavailability, solubility, absorption, distribution, metabolism and excretion (Kelley et al. 2013).

MacFarlane et al. reported that the ionic liquid permeation through membrane depends on the nature of the components. It is well known fact that the salts are less likely to permeate through the membrane from solution. The transport property of pharmaceutically active protic salts namely tuammoniumheptane salicylate and butylammoniumacetate was evaluated via a silicone membrane by applying either propylene glycol solution of IL or by applying neat IL. Two types of protic IL were studied one which is fully dissociated or complete proton transferred while the other protic IL is formed by partial transfer of proton resulting in formation of hydrogen bond (Figure 2.13).

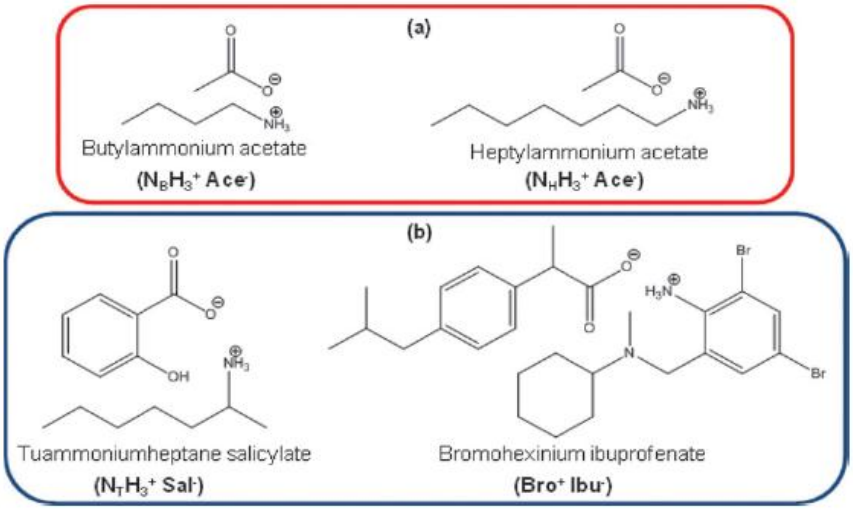


Figure 2.13 Protic ILs (a) proton transferred and fully dissociated (b) proton transferred but form hydrogen bonded clusters (Stoimenovski and MacFarlane 2011)

The hydrogen bonded complexes behave more likely to neutral compounds and function as ion-pair complexes. The protic IL are able to cross the membrane as fast as unionised neutral molecules because they are more associated and fail to exist in solution. On the other hand the starting materials which are simple salts fail to cross the membrane which could be attributed to the general rule that salts do not permeate through the membrane as rapidly as neutral molecules. However the protic ILs which are formed by the complete transference of acidic hydrogen are more likely to be dissociated in the solution or possess very low ability to form associated species also pass through the membrane but not as fast as associated protic ILs (Stoimenovski and MacFarlane 2011).

Liquid co-crystals or deep eutectics are the equimolar ratio of two components which have different properties from those of individual components. A very strong, partially ionized hydrogen bond is responsible for the liquefaction of the crystalline pharmaceuticals. Liquid co-crystals are the unique complexes with

very strong hydrogen bond in which the acidic proton is thought to be localised between the acidic and basic components. A unique example of liquid co-crystal is the combination of lidocaine with fatty acids such as oleic acid or decanoic acid. The formation of such type of complexes results in a partially, not completely, proton transferred (ionized) state of each acid and base (Figure 2.14). Thus specific type of interactions could allow the delivery of active pharmaceutical ingredient transdermally (acidic and basic drug moieties). The strong interaction through acidic proton not only helps to prevent crystallisation of neutral components of the liquid co-crystal but also simultaneous delivery of acidic and basic moieties (Bica et al. 2011).

Figure 2.14 Hydrogen bonded complexes

The mechanism of action of ionic liquid is dramatically influenced by the interactions found between the components. This could be explained by taking a very simple example of lidocaine ibuprofen ionic liquid, which is formed by mixing the two components. Lidocaine ibuprofen has been reported as completely ionized API-IL in some of the publications (Park and Prausnitz 2015), but extensive characterisation of lidocaine ibuprofen illustrates that the combination of these components results in the generation of deep eutectic liquid co-crystals through partially ionized interactions between two components or via strong hydrogen bond (Figure 2.15).

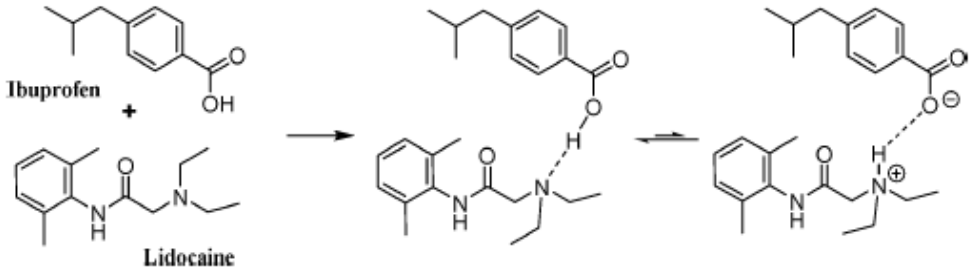


Figure 2.15 Interactions between lidocaine and ibuprofen (Berton et al. 2017)

These interactions of the API-IL are responsible for different biological nature. The bioavailability of the ionized compounds was found to lower because the permeation through membrane of such type of compounds is limited, which is not the case of hydrogen bonded complex's (Lidocaine-ibuprofen) (Berton et al. 2017). These studies suggest that the proper choice of API in its liquid form and comprehensive characterisation of ionic liquids (allows overcoming the misunderstanding of interactions between components of ionic liquids) could have significant influence on the membrane transport properties, an important factor in bioavailability.

2.2.2IL-mediated topical/ transdermal drug delivery

2.2.2.1 IL as carrier for topical/transdermal drug delivery

Studies have been reported which suggest that ILs could be useful for the solubilisation of poorly soluble drugs such as acyclovir, methotrexate and dantrolene sodium. They have reported IL-in-oil microemulsion in which the drug molecules were loaded in the IL droplets and these IL droplets are stabilised by a surfactant and co-surfactant in a continuous phase. A blend of nontoxic surfactant such as polyoxyethylene sorbitan monooleate (Tween 80) and sorbitan laurate (Span-20) were used to prepare IL-in-oil microemulsion. Isopropyl myrisate was used as continuous oil phase. It was observed that the

IL with strong coordinating anions which are strong hydrogen bond accepter can form IL droplets in the oil phase among the studied set of ILs (Figure 2.16). The authors suggested that the interactions between the polar groups of the drug molecule and the IL anion via hydrogen bonding are the key factor responsible for dissolution of sparingly soluble drug molecules. The *in vitro* analysis of skin permeation using full-thickness skin pieces from Yucatan micro pigs showed that the developed IL-in-oil microemulsion displays enhanced release of the drug acyclovir. The increase in the permeability of acyclovir by using IL-in-oil microemulsion was attributed to the high solubilisation of drug molecule in IL droplets and the effect of lipophilic components (Moniruzzaman et al. 2010a).

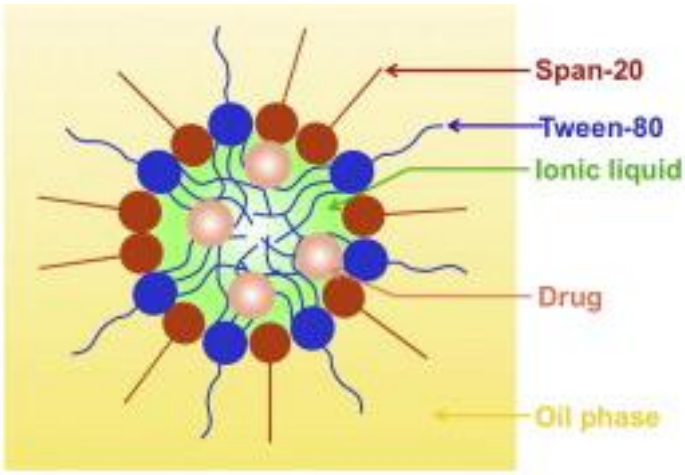


Figure 2.16 Ionic Liquid-in-oil microemulsion (Moniruzzaman et al. 2010a)

A similar study has been reported which illustrates the potential of IL-in-oil microemulsion as an efficient transdermal drug delivery of sparingly soluble drugs. The developed formulation based on microemulsion was shown to increase the administration of MTX which was used as model drug. DMSO, a well-known chemical enhancer used for transdermal or topical drug delivery is

safe only when it is used in very low concentration (up to 10%). Cytotoxicity of the system was remarkably increased in the presence of highly concentrated DMSO. The same pattern was followed by the authors where they developed the IL-in-oil microemulsion having low concentration of ILs and they observed an increase in cell viability (> 80%) of a cancerous cell when compared with the controls (Dulbecco's phosphate-buffered saline, water-in-oil microemulsion and IPM) (Yoshiura et al. 2013) .

Another study suggests the subsequent development of a novel IL-in-oil microemulsion system for the dermal delivery of water soluble (poorly permeating) BCS class III drug 5-fluorouracil. An imidazole based ionic liquid 1-butyl-3-methyimidazolium bromide (BMIM Br) was used for development of microemulsion. The authors observed remarkable enhancement in the solubility of 5-flurouracil in IL-in-oil microemulsion as compared to water-in-oil microemulsion. IL-in-oil microemulsion was developed by using IPM, Tween 80, Span 20 and [BMIM] [Br]. *Ex-vivo* skin permeation studies were also conducted through mouse skin and the results indicates that the developed IL-in-oil microemulsion induced the significant permeation of 5-flurouracil. For example, when compared with the aqueous solution, hydrophilic ointment and water-in-oil microemulsion shows 4-fold, 2.3-fold and 1.6-fold respectively enhancement in the percent drug permeation. In addition to that the developed IL-in-oil microemulsion formulation could be effectively used to treat skin cancer. The analysis of *in-vivo* studies against dimethylbenz(a)anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mice skin carcinogenesis illustrates that IL-in-oil microemulsion could treat skin cancer effectively. Importantly erythema and irritation (common side effects) which were associated with the conventional drug formulations were not observed.

Interestingly, the histopathological studies suggest that the utilisation of the IL-in-oil microemulsion did not cause any pathological and anatomical changes in the skin structure of the mice (Goindi et al. 2014).

Later, in a similar study, the influence of hydrophilic [HMIM] [Cl] and hydrophobic [BMIM] [PF6] imidazolium based ionic liquids on the properties and stability of emulsion was investigated. In this study the water phase was replaced by [HMIM] [Cl] and the oil phase replaced by [BMIM] [PF6]. Both ionic liquids were successfully incorporated into the emulsion structure, resulting in stable formulations. Both of the ILs could be used as preservatives in the emulsion as they show antimicrobial activity. The droplet size and viscosity of the IL-based formulation was remarkably decreased and also maintained long term stability as compared with emulsion without IL. *In-vitro* cytotoxicity evaluation of the formulation containing hydrophilic and hydrophobic ILs suggests low cytotoxicity of the carriers. In addition to that skin penetration enhancement of the fluorescent dyes as a model drug was achieved in presence of ILs(Dobler et al. 2013).

Goindi et al. (2015), reported the potential application of IL-in-water microemulsion formulation which is able to solubilize a poorly water soluble anti-inflammatory drug etodolac (ETO). The microemulsion formulation is made up of IL [BMIM] [PF6], while tween 80 and ethanol was used as surfactant and co-surfactant. The *ex-vivo* drug permeation evaluation shows that IL-in-water microemulsion was effective in enhancing the drug solubility and shows better skin permeation when compared with other studied formulations. The *in-vivo* anti-arthritic and anti-inflammatory activities also suggested that ETO loaded IL-in-water microemulsion was found to be more effective in controlling inflammation compared with commercial formulation of ETO and the oily

formulation, o/w microemulsion. The histopathological studies illustrate no anatomical and pathological changes in skin (Goindi et al. 2015).

Araki et al. (2015) reported the potential application of ionic liquid as penetration enhancer in the drug delivery systems. In this study the ionic liquid [C12MIM][NTf2] was incorporated in as penetration enhancer in the studied vaccine formulations, resulting in significant enhanced skin-permeating ability of ovalbumin (OVA), a model antigen. The study illustrates that IL-mediated transcutaneous administration of high molecular weight protein and drug molecules was effective using solid-in-oil nanodispersions (Figure 2.17) (Araki et al. 2015).

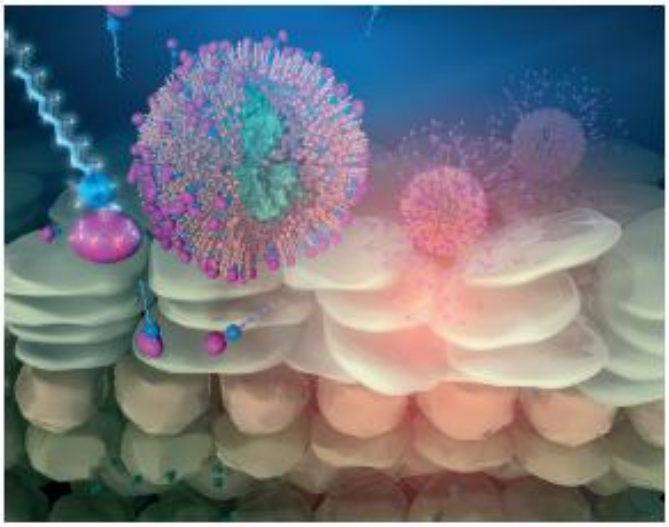


Figure 2.17 Transcutaneous protein delivery using solid-in-oil nanodispersions (Araki et al. 2015)

Koji Kubota et al. (2016) reported the formation of molecular assembly by combination of highly biocompatible and safe carboxylic acid and aliphatic amines, which was studied for an effective and safe penetration enhancer for transdermal drug permeation. Hydrophilic phenol red and hydrophobic tulobuterol was used to understand the mechanism of skin permeation

enhancement using the ionic liquids. FTIR was used to characterise the IL mixture which suggest that the carboxylic acid/amine mixture coexists at all states of mixture. ILs interact with the carboxylic acid via hydrogen bonds when acid is in excess state, thus the skin permeation enhancement effect was not only due to presence of ionic liquid (Kubota et al. 2016).

2.2.2.2 Drug-IL for topical/transdermal drug delivery

Lidocainium docusate was the very first API-IL made by Rogers and Houh et al. (2007). The author's studied the topical treatment of analgesia in the two different models of mouse anti-nociception and observed remarkable enhancement compared with the parent API, lidocaine hydrochloride. They also observed the significant increase in the physical properties such as thermal stability and solubility of the synthesised salt [lidocainium] [docusate] (Hough et al. 2007a).

Zakrewsky et al studied a set of neat ILs for the enhancement of the antibiotic delivery across skin layers. Choline geranate [Chol] [Geranate] was found be effective among all the studied neat ILs. This IL shows minimal toxicity against several human cancer cell lines. Choline geranate effectively enhanced the skin permeation for the delivery of the antibiotic cefadroxil by a factor of > 16 without causing skin irritation. Importantly, in-vivo evaluation suggests outstanding antimicrobial activity against the biofilm protected microbes *pseudomonas aeruginosa and salmonella enterica*, greater than 95% bacterial death was observed after 2 hour treatment in a biofilm-infected wound model (Zakrewsky et al. 2014).

Wang et al. (2014) have shown that acidic and basic active pharmaceutical ingredients (API) can be held strongly in deep eutectic form (liquid co-crystal) through hydrogen bonds or partially ionized interactions even in solution form.

In order to demonstrate this, researchers use permeation via a model membrane in a Franz diffusion cell and illustrate that acidic and basic APIs transports at much higher rate than the solutions of the individual material. By just mixing the solvent and corresponding API solutions, hydrogen bonded complexes are formed which are responsible to form deep eutectic of active pharmaceutical ingredients *in situ.* Therefore it is not necessary to premade deep eutectic of active pharmaceutical ingredients. In order to understand the nature of interactions between acidic and basic API, a number of nonstoichiometric mixtures of lidocaine and ibuprofen has been studied via spectroscopically and membrane transport (Wang et al. 2014).

The potential application of pharmaceutically active IL has been demonstrated by all- in-one concept for drug delivery systems. An ionic liquid has been synthesised by combining the cationic drug amitriptyline hydrochloride (AH) and anionic surfactant molecule sodium dodecyl sulphate (SDS) and characterised by ^{1}H NMR and DSC. The newly formed ionic liquid salt could work in both ways it can act as carrier and as well as the active drug molecule and in aqueous solution can undergo self-assemble to form vesicles (Figure 2.18). Controlled release of the drug molecule was observed from the carrier. The ionic liquid strategy of synthesising such type of pharmaceutically active IL vesicles offers many attractive advantages over conventional liposomal or polymer based drug delivery systems. For instance, fixed or high drug loading could be achieved up to 57.7%, depending on the equivalent mole number of SDS^{-} and AH^{+}. The method of preparation of pharmaceutical IL vesicles is quite simple, easy and the pharmaceutical IL is more stable. The newly prepared ionic liquid displays enhanced permeability compared to the drug component in the ionic liquid (Zhang et al. 2013).

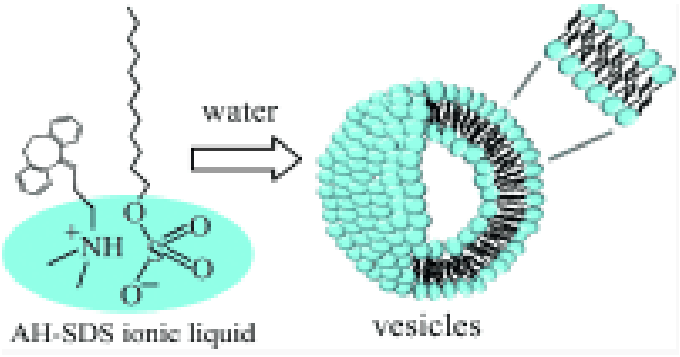


Figure 2.18 Displaying self-assembling of API-IL (Zhang et al. 2013)

Cholinium sulfasalazine (IL) was evaluated for its pharmacological performance as it has very low membrane permeability activity and limited water solubility (0.6 µg ml^{-1}). Due to the poor exposure of the drug, a frequent oral dose of 1-3 g per day is required by the arthritis patents. The synthesised ionic liquid salt (cholinium sulfasalazine) was found to exhibit enhanced solubility profile compared to parent drug. *In-vivo* studied were conducted using rats which were exposed to intravenous (I.V.) administration. Interestingly, the synthesised salt was found to be administered in much higher I.V. doses compared to the low solution dose achieved by the physical mixture (Sulfa : choline) (Shadid et al. 2015).

Park and Prausnitz (2015) reported lidocaine (a local anaesthetic) in the ionic liquid form, which allows enhanced lidocaine absorption into the skin and reduce the time to achieve local anaesthesia. The idea behind this study was that the injection process to administer the local anaesthesia lidocaine was painful and not comfortable. In addition to that the eutectic mixture of local anaesthesia, which is equal quantities of lidocaine and prilocaine and is marketed as a 5% oil-in-water emulsion incorporated in a cream need to apply before 1 hour in order to achieve local anaesthesia. To overcome these

shortcomings authors performed a number of screenings with different counterions. Interestingly, they manage to get ionic liquid form of lidocaine which is synthesised using ibuprofen. This study reports the potential of lidocaine-ibuprofen ionic liquid as improved and rapid method of topical drug delivery and results indicate enhanced lidocaine absorption into skin (Park and Prausnitz 2015).

The development of ionogels as drug delivery system has been explored by Lydie et al. Ionogels are prepared in two steps, first step is to made 1-methyl-3-butylimidazolium ibuprofenate (IL) by ion exchange reaction carried between sodium salt of ibuprofen and 1-methyl-3-butylimidazolium chloride. Then the IL synthesised in first step is used in sol-gel synthesis using pure tetramethoxysilane (TMOS) or tetramethoxysilane/ methyltrimethoxysilane (MTMOS) precursor mixtures. Ionogels were characterised by FTIR and XRD. Results of this study illustrates that the kinetics of the drug releasing system was controlled by the nature of the silica wall (Viau et al. 2010).

In a recent study, a poorly soluble drug, etodolac has been converted into ionic liquid by mixing it with lidocaine in stoichiometric proportions in order to enhance its hydrophilicity, hydrophobicity and skin permeability. Differential scanning calorimetry, FTIR, and saturation measurement were used to characterise the synthesised ionic liquid and the parent drug molecule. The newly formed ionic liquid exhibit lower melting point compared to corresponding individual drug molecule alone which confirms the enhancement in lipophilicity/hydrophilicity of etodolac. *In-vitro* assessment has been carried out through patch (Etoreat) containing ionic liquid and the results show significantly enhancement in skin permeation of etodolac (9.3 fold) compared to etodolac patch alone. However no significant change was observed for lidocaine skin

permeation. This study illustrates that lidocaine substantially expand the formulation and helps to improve skin permeation of etodolac (Miwa et al. 2016).

Berton et al. (2017) evaluated the bioavailability of lidocaine on the basis of the interaction found in the formulation. The study begins with the incorporation of lidocaine to produce ionic liquid salt lidocainium docusate and lidocaine-ibuprofen (deep eutectic). The transdermal absorption of lidocainium docusate and lidocaine-ibuprofen (deep eutectic) was compared with crystalline salt lidocainium chloride. Topical creams of crystalline salt lidocainium chloride and the salt and deep eutectic were prepared by mixing each compound to the vehicle cream to meet the concentrations of 3.5 and 5.0 wt % of lidocaine to mimic commercially available formulations. Then these topical creams were applied to Sprague-Dowley rats. The plasma concentration of lidocaine was monitored over time. Interestingly, the concentration of lidocaine in plasma was found to higher and faster in hydrogen bonded deep eutectic lidocaine ibuprofen compared to lidocainium chloride and lidocainium docusate (Figure 2.19) (Berton et al. 2017).

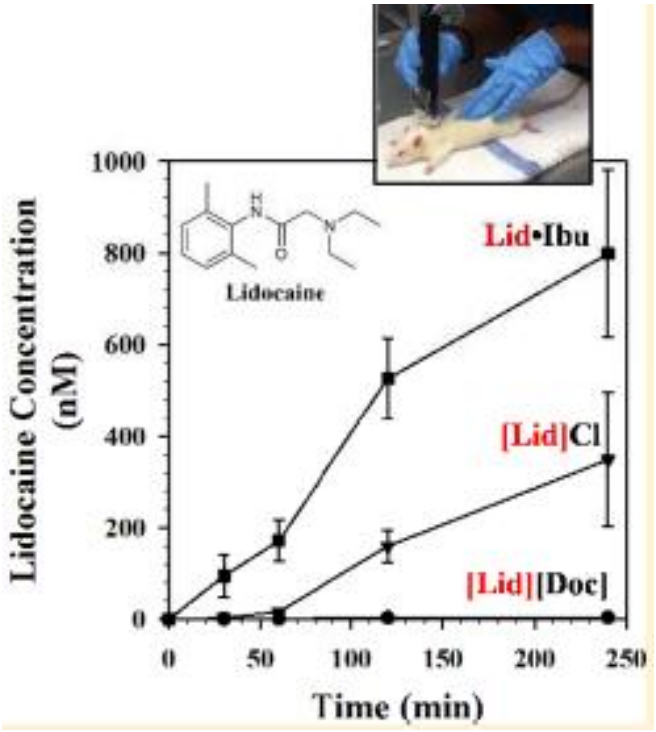


Figure 2.19 Concentration of lidocaine in blood plasma over time after the application of creams (Berton et al. 2017)

2.2.3 Summary of literature review

The literature review covers the detailed studies relevant to the potential application of ILs in the field of topical and transdermal drug delivery. So the next section provides detailed information regarding materials used for synthesis, methods and different analytical techniques used for the study.

Chapter 3 Materials, methods and general experimental procedures

3.1 Introduction

This chapter describes the materials and methods used for synthesis of benzalkonium based NSAIDs ILs, benzalkonium-sulfacatmide ILs, benzalkonium based mixed anion ILs and ibuprofen IL hydrogels. This section also provides detailed information regarding experimental procedures and analytical tools such as NMR and IR used for characterisation.

3.2 Materials

3.2.1Chemicals used for the synthesis of benzalkonium based NSAIDs ILs

Benzyltriethylammonium chloride, benzyldimethylhexadecylammonium chloride, sodium ibuprofen, chloroform, methanol, acetic acid, acetonitrile, 1-octanol were purchased from Sigma –Aldrich. Sodium diclofenac was purchased from VWR UK.

3.2.2 Chemicals used for the synthesis of benzalkonium-sulfacetamide ILs

Benzyltriethylammonium chloride, benzyldimethylhexadecylammonium chloride, sodium sulfacetamide, chloroform, methanol, acetic acid, acetonitrile, 1-octanol were purchased from Sigma –Aldrich.

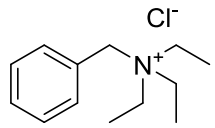
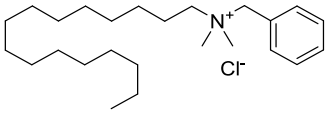
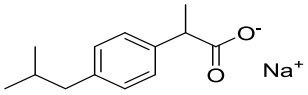
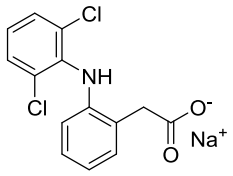
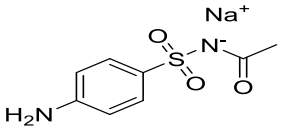
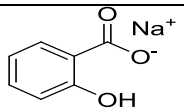
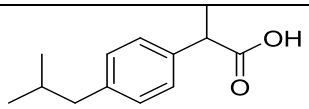
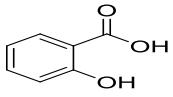
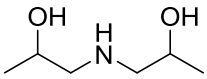
3.2.3 Chemicals used for the Synthesis of benzalkonium based mixed anion ILs

Benzyltriethylammonium chloride, benzyldimethylhexadecylammonium chloride, salicylic acid, sodium salicylate, sodium ibuprofen, ibuprofen, chloroform, methanol, acetic acid, acetonitrile, 1-octanol were purchased from Sigma –Aldrich.

3.2.4 Chemical used for synthesis of diisopropanolamine-ibuprofen (IL) and for formulation containing IL hydrogels

Carbopol 974 was purchased from Lubrizol, diisopropanolamine, dialysis tubing cellulose membrane size 33mm × 21 mm were purchased from Sigma Aldrich. Distilled deionized water was used for making gels. Dura pore membrane filters, and Filter type: 0.45 µm HVHP were purchased from Sigma –Aldrich.

Table 3.1 Physicochemical properties of used materials

Name	Structure	Molecular weight	Melting point (°C)	Source	Purity (%)
BTEA-Cl		227.77	190-192	Sigma	99
BDMA-Cl		396.09	55-56	Sigma	99
Ibuprofen sodium		228.26	129-131	Sigma	98
Diclofenac sodium		318.13	-	VWR	98
Sulfacetamide sodium		254.24	-	Sigma	99
Salicylic sodium		160.10	200-202	Sigma	98
Ibuprofen		206.28	76-78	Sigma	98
Salicylic acid		138.12	158-161	Sigma	99
Diisopropanola -mine		133.19	42-45	Sigma	95

3.3 Methods used for synthesis

3.3.1 Synthesis of benzalkonium based NSAIDs ILs

Ionic liquids were synthesised according to previously reported methods (Hough-Troutman et al. 2009; Rogers et al. 2014). Four benzalkonium based NSAIDs ILs have been synthesised using a common synthesis (Figure 3.1):

benzyltriethylammonium-ibuprofenate,

benzyltriethylammonium-diclofenac,

benzyldimethylhexadecylammonium-ibuprofenate,

benzyldimethylhexadecylammonium -diclofenac.

A common procedure was followed to synthesize the above listed API-ILs in the purest form using commercially available starting materials (Figure 3.1). Each of the cations was combined with ibuprofen and diclofenac anions by a stoichiometric metathesis reaction in aqueous solution using the sodium salt of each drug. The nature of the salts allowed them to be easily extracted from the aqueous phase into chloroform, which was then washed with water to remove the inorganic salt (monitored by silver nitrate test). Finally the solvent was removed by a rotary evaporator and it was dried under vacuum.

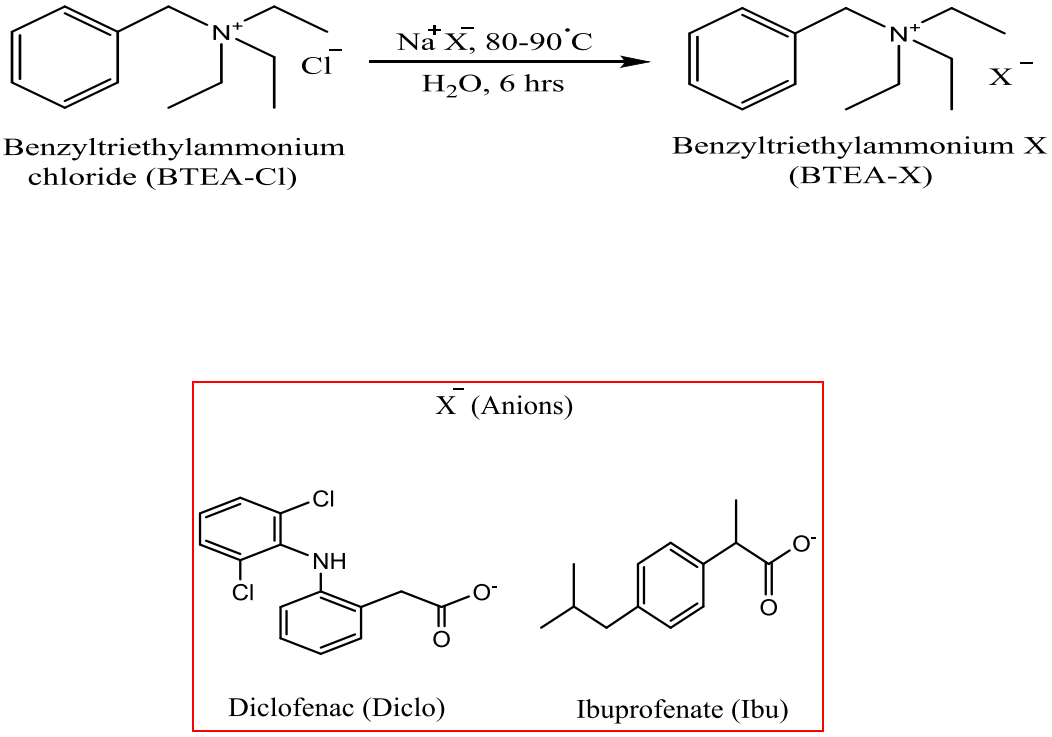


Figure 3.1 Scheme for the preparation of benzalkonium based NSAIDs ILs

3.3.2 Synthesis of benzalkonium sulfacetamide ILs

Benzyltriethylammonium sulfacetamide and Benzyldimethylhexadecylammonium sulfacetamide were synthesised using common synthesis process (Figure 3.2), which is explained in 3.3.1 section.

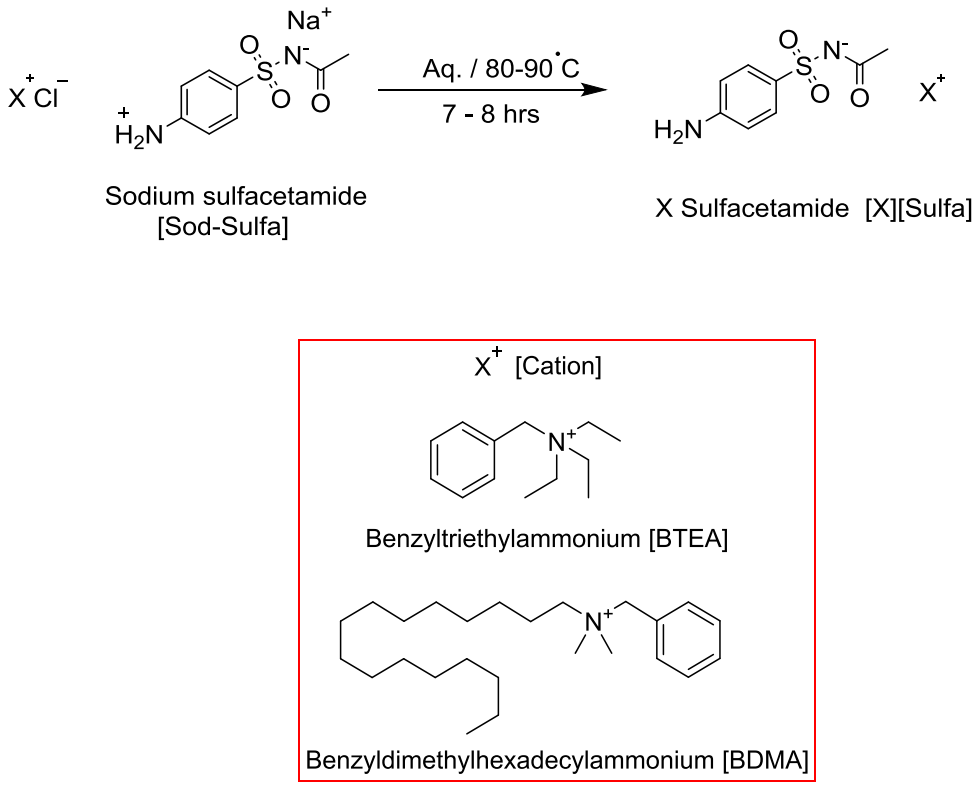


Figure 3.2 Scheme for the preparation of benzalkonium-sulfacetamide ILs

3.3.3 Synthesis of benzalkonium based mixed anion ILs

Four benzalkonium based mixed anion ILs have been synthesized: benzyltriethylammonium-ibuprofenate salicylic acid, benzyltriethylammonium-salicylate ibuprofen, benzyldimethylhexadecylammonium-ibuprofenate salicylic acid, benzyldimethylhexadecylammonium –salicylate ibuprofen.

A two-step common procedure was followed to synthesize the above listed ILs with mixed anions using commercially available starting materials (Figure 3.4).

1. Four benzalkonium based NSAIDs ILs namely benzyltriethylammonium-ibuprofenate, benzyltriethylammonium-salicylate, benzyldimethylhexadecylammonium-ibuprofenate and benzyldimethylhexadecylammonium-salicylate were synthesised using common synthesis process, which is explained in Section 3.3.1.

2. The benzalkonium based NSAIDs ILs synthesized in step first was mixed with ibuprofen/salicylic acid to give ILs with mixed anions using conventional and solvent free approach:

(A) Conventional method:

Benzyltriethylammonium ibuprofenate and solid salicylic acid in equimolar ratio were dissolved in 20 ml methanol and stirred for 30 minutes at room temperature. The solvent was evaporated to obtain benzyltriethylammonium-ibuprofenate salicylic acid as a clear viscous liquid. The purity was confirmed by ^{1}H NMR, ^{13}C NMR, DSC and TGA.

(B) Solvent free synthesis:

Benzyltriethylammonium ibuprofenate and salicylic acid in equimolar ratio were ground for 25 minutes in a mortar at room temperature. A viscous liquid was obtained**.** Theremainingbenzalkonium ILs with anions were synthesised using the common synthesis method and were confirmed by ^{1}H and ^{13}C NMR, TGA and DSC. All the remaining ILs with mixed anions have been synthesised by common synthesis (Table3.1).

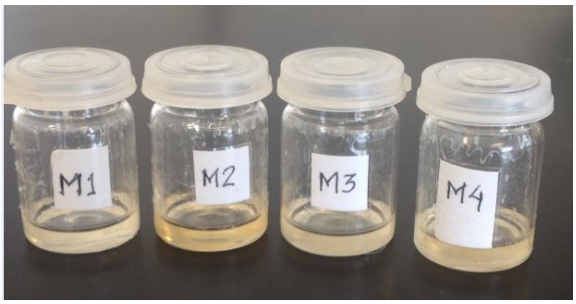


Figure 3.3 Photograph showing benzalkonium based mixed anion ILs

Table 3.1 Benzalkonium based mixed anion ILs

Sample ID	Benzalkonium based NSAIDs ILs	Acid	API-IL: Acid (Ratio)	Benzalkonium based mixed anion ILs
M1	Benzyltriethylammonium ibuprofenate [BTEA-Ibu]	Salicylic acid	1:1	Benzyltriethylammonium ibuprofenate-salicylic acid
M2	Benzyltriethylammonium Salicylate [BTEA-Sal]	Ibuprofen	1:1	Benzyltriethylammonium salicylate-Ibuprofen
M3	Benzyldimethylhexadecylammonium Ibuprofenate [BDMA-Ibu]	Salicylic acid	1:1.5	Benzyldimethylhexadecylammonium ibuprofenate-salicylic acid
M4	Benzyldimethylhexadecylammonium Salicylate [BDMA-Sal]	Ibuprofen	1:1.5	Benzyldimethylhexadecylammonium Salicylate- Ibuprofen

*API-IL: Active pharmaceutical ingredients-ionic liquid

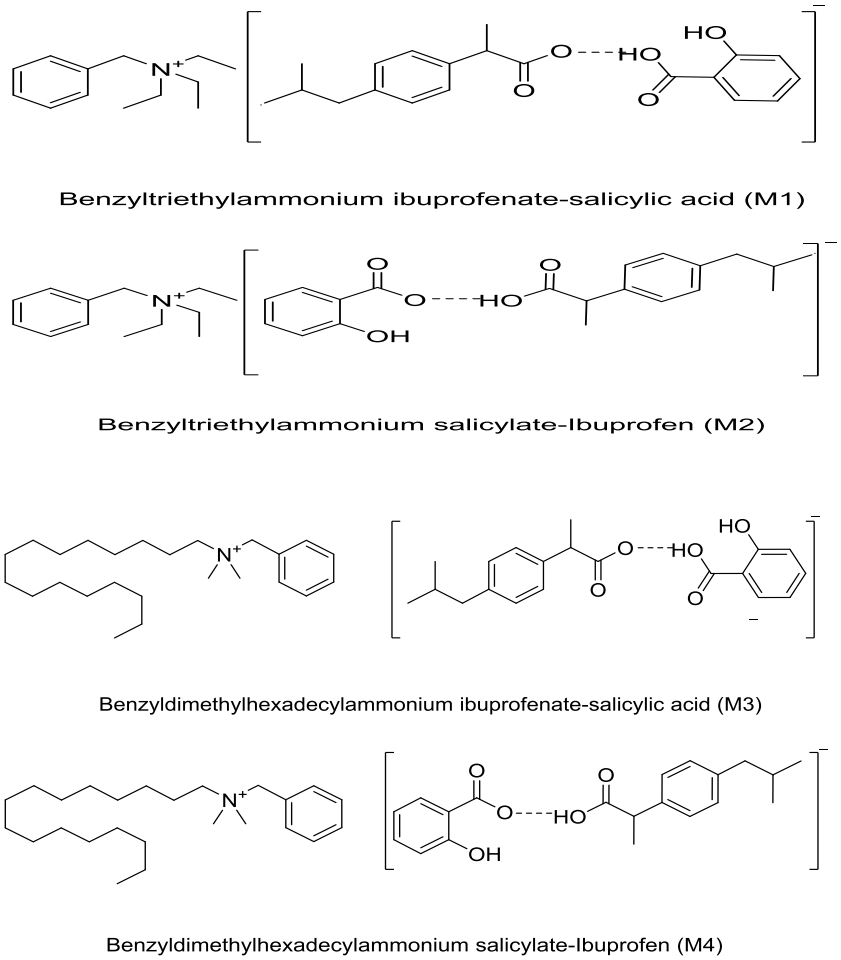


Figure 3.4 Representing structural formulae of benzalkonium based mixed anion ILs

3.3.4 Synthesis of synthesis of diisopropanolamine-ibuprofen (IL) and formulation containing IL hydrogels

3.3.4.1 Synthesis of synthesis of diisopropanolamine-ibuprofen (IL)

Appropriate amounts of Ibuprofen and diisopropanolamine were charged in a 50 ml beaker and heated to a temperature in the range of 80-90ºC for 30-40 minutes to form a homogeneous colourless liquid which was characterised by ^{1}H NMR, ^{13}C NMR, and FTIR (Figure 3.5). The compositions of the ILs are shown in Table 3.2

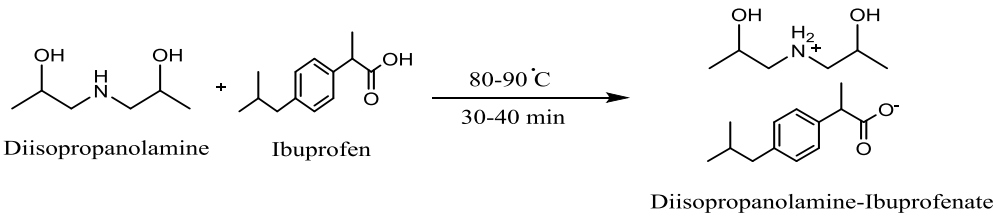


Figure 3.5 Scheme for synthesis of diisopropanolamine-ibuprofen (1:1)

Table 3.2 The various compositions of diisopropanolamine-ibuprofen IL

Sample.	Ratio DIPA-Ibu* (IL)	Amount of DIPA (g)	Amount of Ibuprofen (g)	Physical observation
DI* 0.2	0.2:1	0.128	1	Slightly hazy,liquid
DI 0.4	0.4:1	0.257	1	Colourless, uniform liquid
DI 0.5	0.5:1	0.322	1	Colourless, uniform liquid
DI 0.6	0.6:1	0.386	1	Colourless, uniform liquid
DI 0.8	0.8:1	0.515	1	Colourless, uniform liquid
DI 1	1:1	0.644	1	Colourless, uniform liquid
DI 1.2	1.2:1	0.778	1	Colourless, uniform liquid
DI 1.4	1.4:1	0.902	1	Colourless, uniform liquid
DI 1.5	1.5:1	1.018	1	Colourless, uniform liquid
DI 1.6	1.6:1	1.031	1	Colourless, uniform liquid
DI 1.8	1.8:1	1.160	1	Colourless, uniform liquid
DI 2	2:1	1.289	1	Colourless, uniform liquid

DIPA-Ibu* (IL): Diisopropanolamine-Ibuprofen ionic liquid

DI*: Diisopropanolamine-Ibuprofen

3.3.4.2 Formulation of IL based ibuprofen hydrogels

IL based ibuprofen hydrogels were prepared by incorporating 1% aqueous carbopol dispersion into synthesized diisopropanolamine-ibuprofen (IL) according to the following steps:

a) 1 g of Carbopol 974 powder was dispersed in a final volume of 100 ml deionized water and left overnight to form 1% aqueous carbopol homogeneous dispersion.

b) This 1% aqueous carbopol dispersion was subsequently added to the various synthesized IL compositions.

c) The mixtures obtained in step (b) were stirred gently with the help of a spatula until a homogeneous hydrogel was formed. The pH of the IL based ibuprofen hydrogels was measured by Mettler Toledo pH meter.

d) The IL based ibuprofen hydrogels obtained in step (c) were allowed to equilibrate for at least 2 days at room temperature and were then characterised by FTIR. The compositions of the IL based ibuprofen hydrogel formulation are shown in Table 3.3.

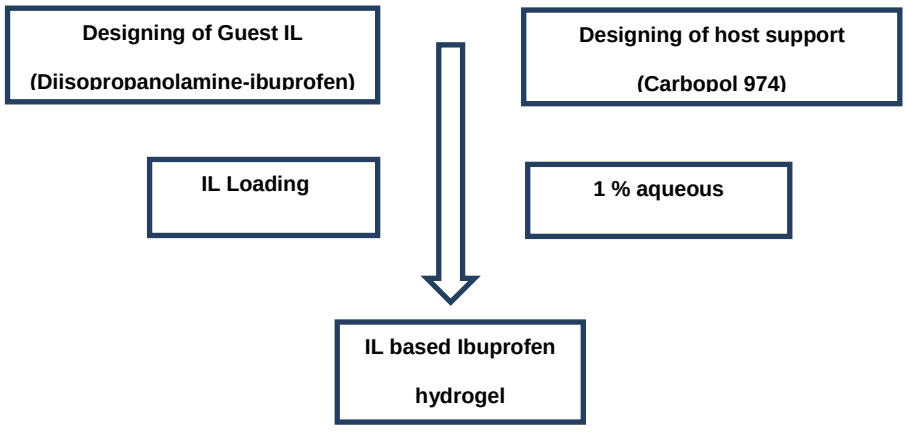


Figure 3.6 Diagrammatic depiction of the method of preparation of IL based ibuprofen hydrogels

Table 3.3 Compositions of IL based ibuprofen hydrogels

Sample	Amount of Un-neutralised 1% carbopol dispersion	pH of Un-neutralised 1% carbopol dispersion	Ratio DIPA*:Ibuprofen (IL)	Amount of DIPA*:Ibuprofen (IL) (g)	pH of gel	Strength of gel	Physical observation
S1	25 g	3.02	1:1	1.644	7.45	4 %	Cloudy, uniform gel
S2	25 g	3.02	1.2:1	1.778	7.48	4 %	Cloudy, uniform gel
S3	25 g	3.02	1.4:1	1.902	7.54	4 %	Cloudy, uniform gel
S4	25 g	3.02	1.5:1	2.018	7.70	4 %	Colourless, uniform gel
S5	25 g	3.02	1.6:1	2.031	7.75	4 %	Colourless, uniform gel
S6	25 g	3.02	1.8:1	2.160	7.88	4 %	Colourless, uniform gel
S7	25 g	3.02	2:1	2.289	8.7	4 %	Colourless, uniform gel

*- DIPA-Diisopropanolamine

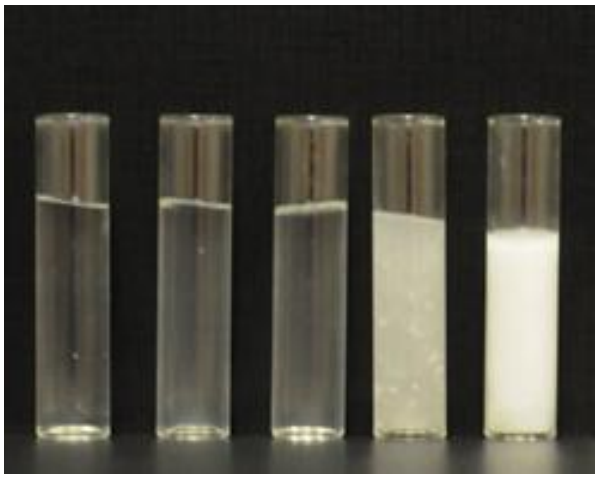


Figure 3.7 Photograph of the prepared IL based ibuprofen hydrogels (right to left) [S2], [S3], [S4], [S5], [S6]

3.4 General experimental procedures and analytical techniques

3.4.1 ^{1}H and ^{13}C NMR Spectroscopy

NMR spectra of samples were obtained using a Bruker Avance III 400 spectrometer operating at 400 MHz, in $CDCl_3$. Tetramethylsilane was used as internal standard. The ^{1}H NMR spectra were recorded in the range of 0-20 ppm at ambient probe temperature (273 K) at 16 scans. Coupling constants are quoted in Hertz (Hz). In general the sample size used was 10-20 mg and 0.7 ml deuterated chloroform

^{13}C NMR spectra were recorded in the range of 0-250 ppm at 128 scans.

3.4.2 FTIR Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR): Infrared spectra were recorded using neat samples on a Perkin-Elmer Nicolet FTIR spectrometer. Spectra were obtained in the range of 400-4000 cm^{-1}

3.4.3 Differential Scanning Calorimeter

Thermal transitions (melting point and glass transitions) of the neat samples were determined on a TA Instruments DSC 2000 differential scanning calorimeter. Approximately 5-10 mg of the sample was placed in a standard aluminium pan with a pin-hole in the lid. The instrument was calibrated with indium and zinc standards. The samples were subject to heat-cool-heat cycle. The samples were first equilibrated at 10 ˚C, then heated to 80 ˚C (heating rate 10 °C/min) and then held for 5 minutes at 80 ˚C. After this, samples were cooled to -70 ˚C (cooling rate 10 °C/min) and held for 5 minutes and then again heated to 80 ˚C.

3.4.4 Thermogravimetric analysis

Thermogravimetric analysis was performed to determine the range of thermal decomposition temperatures for each of the synthesized ionic liquids. The study was performed using TA Instruments Q5000 series thermal gravimetric analyzer. The sample (5-10 mg) was loaded in a platinum pan and heated from 25 ˚C to 350 ˚C at a heating rate of 10 ˚C min^{-1} under inert nitrogen environment. The obtained data was analyzed using TA Universal analysis software V4.5A.

3.4.5 Electrical conductivity measurement

The conductivity measurements were carried out on YSI Professional plus portable multiparameter water quality meter. The instrument was calibrated before and after use by using 1M KCl solution. The instrument conductivity ranges from 0 to 50 mS/cm. All the measurements were carried out at room temperature were repeated three times, and the averages of the values were taken.

3.4.6 Determination of octanol-water partition coefficient

The octanol-water partition coefficient of ionic liquids was determined using a procedure mentioned by Zakrewsky et al. (2014). Firstly, octanol was saturated with water. 250 mL of n-octanol was mixed and shaken with 100 mL of distilled water and left overnight. The saturated octanol was used for the preparation of 0.01 M solutions of each ionic liquid in a 5-mL volumetric flask. In addition 0.01-M solutions of each benzalkonium based NSAIDs ILs were also prepared using water. Separately, saturated octanol and phosphate buffer pH 7.2 was used for the preparation of 0.01 M solutions of M1, M2 and 0.0005 M solution of M3, M4 ILs (benzalkonium based mixed anion ILs) in a 5-mL volumetric flask. Absorbance maxima for benzalkonium based NSAIDs ILs and benzalkonium

based mixed anion ILs are provided in Table 3.5. A 4-mL portion of the IL solution in saturated octanol was shaken with 4 mL of distilled water/phosphate buffer and this was followed by gentle centrifugation (1,000 rpm, Hettich zentrifugen LBA centrifuge, 6-hole fixed angle rotor 804SF) to obtain clean separation of the two layers. The UV absorbance of the octanol and water/phosphate buffer layers was measured and compared with the absorbance of the stock solutions. Measurements were repeated three times, and the partition coefficients were reported as the average. The percentage of ionic liquid in octanol was calculated as the absorbance of the octanol layer after extraction divided by the absorbance of ionic liquid in octanol before extraction. The water/octanol distribution coefficient was calculated as the logarithm of the percentage of IL in octanol divided by the percentage of IL in water(Zakrewsky et al. 2014).

Table 3.4 Absorption maxima for benzalkonium based NSAIDs ILand benzalkonium based mixed anion ILs

S.no	Benzalkonium based NSAIDs ILs	Benzalkonium based mixed anion ILs	Absorption maxima
1	[BTEA] [Ibu], [BDMA] [Ibu]	-	263 nm - 264 nm
2	[BTEA] [Diclo], [BDMA] [Diclo]	-	286 nm- 287 nm
3	[BTEA] [Sulfa], [BDMA] [Sulfa]	-	257 nm – 258 nm
4	-	M1, M2, M3, M4	296 nm - 297 nm

3.4.7 Ex-vivo skin study

Rat skin was carefully cleaned under cold running water and stored at -20ºC before use. On the day of the experiment, the skin was defrosted, cut into square pieces, and mounted with the stratum corneum uppermost in Franz-type diffusion apparatus (Hathout and Nasr 2013; Bsieso et al. 2015; Nasr and Abdel-Hamid 2016; Abdelgawad et al. 2017; Nasr et al. 2017). The area of the

skin was 1.77cm^2. The receptor medium was composed of 7.5 ml phosphate buffer pH 7.4 containing 0.5% tween 80, which was constantly stirred with a magnetic bar, and maintained at 37ºC using a circulating water jacket. An amount of 100 mg of the ionic liquid samples were placed in donor compartments (n=5). Samples were withdrawn at time intervals (15 min, 30 min, 1hr, 1.5 hr, 2 hr, 3 hr, 4 hr, 5 hr) from the receptor compartment, and replaced with fresh medium. After 5 hours, the skin samples were washed five times with distilled water and dried with filter paper to remove any excess formula. Tape stripping of the skin was performed with an adhesive tape, in which twenty pieces of adhesive tape were firmly pressed on the skin surface and rapidly pulled off with fluent strokes. The dermis was separated from the epidermal layer in each skin sample using a scalpel, and the tape strips, dermis and epidermis were individually placed in beakers containing 10 ml methanol each for extraction of the drugs, after placing them in a sonicator for 2 hours. All samples were filtered using a membrane filter of pore size 220 nm before injection into a HPLC column to be analyzed for the drug contents. The amounts of drugs accumulating in the different skin layers and permeated through the skin were expressed as a percentage of the total amount of active ingredient applied on the skin.

3.4.8 In-vitro permeation studies

The *in-vitro* permeation studies of benzalkonium based mixed anion ILs and IL based ibuprofen hydrogels were carried out using a Franz diffusion cell (Copley scientific, vertical diffusion tester) using artificial membrane (Durapore membrane filters, Filter type: 0.45 µm HVHP). Six mL phosphate buffer pH 7.2 was used as receptor chamber and then 200 mg of neat benzalkonium based mixed anion ILs/IL based ibuprofen hydrogels was placed evenly on the

membrane in a thin layer which is used as donor phase. The donor compartment was kept in contact with the receptor chamber and the temperature was maintained in the range of 36 °C - 37 °C and the solutions were stirred by magnetic bars. 3 mL sample from the receptor compartment was taken at appropriate time intervals and replaced by same concentration fresh phosphate buffer solution to maintain sink conditions. Then the samples were analysed by High Performance Liquid Chromatography (HPLC).

3.4.9 In-vitro release studies

Dialysis bags of cellulose membrane were made providing cylindrical compartments for hydrogels and which were washed in water previous to use. The dialysis bags were filled with 0.5 g of the IL based Ibuprofen hydrogels. The exposed surface area of the hydrogel in the dialysis bag was in the range of 3 - 3.5 cm. Then the bags were immersed in the acceptor compartment containing 200 mL of the receptor media, which is phosphate buffer pH 7.2. The temperature was kept in the range of 36 °C -37 °C and the receptor media was constantly stirred with a magnetic bar to maintain sink conditions. At appropriate time intervals a 3 mL sample was taken from the receptor media and replaced by an equal amount of fresh phosphate buffer pH 7.2 to maintain sink conditions. The amount of Ibuprofen diffused into the receptor chamber was analysed by High Performance Liquid Chromatography (HPLC).

3.4.10 Antibacterial efficacy study

3.4.10.1 Materials

Tryptic Soy Agar (TSA), Tryptic Soy Broth (TSB), Müller Hinton agar (MHA); Oxoid Microbiology Products, deionized water, Whatman grade AA discs, 6 mm diameter (Fisher Scientific)

3.4.10.2 Bacterial strains used in the study

Two strains were used:

Staphylococcus (S.) aureus (NCTC 6571)

Escherichia (E.) coli (NCCTC 12923)

3.4.10.3 Culture media and incubation conditions

Culture media was prepared and treated according to manufacturer's guidelines and sterilised by autoclaving. The strains were routinely grown on TSA agar, and incubated at 37 ºC in air for 24 hrs.

3.4.10.4 Screening for antibacterial activity by disc diffusion tests

The *in vitro* antibacterial efficacy of the benzalkonium-sulfacetamide ILs and the individual components were tested against both gram-positive and gram-negative bacteria and in particular against the *Staphylococcus (S.) aureus* (NCTC 6571) *and Escherichia (E.) coli* (NCCTC 12923) *strains* using the agar diffusion method. Bacterial suspensions were prepared from the TSA culture plates by transferring 2-3 colonies in sterile distilled water. The turbidity of the bacterial suspensions was adjusted to 0.5 McFarland turbidity standard, that corresponds to $1.5x10^8$ colony forming units (CFUs)/ml. This bacterial suspension was used to inoculate MHB agar plates evenly using a sterile swab.

2 mg/ml of each test compound was prepared in distilled water and then diluted to the desired concentration range (20, 30, 40, 50 and 100 µg/ml). Then 20 µl of

each tested compound was loaded from the working stock solution onto 6 mm diameter sterile Whatmann discs (Fischer scientific, UK). The loaded discs were placed on the surface of the agar lawns with the help of needle and forceps. The plates were incubated at 37 °C for 24 hours. At the end of the incubation, the plates were examined and the diameter of each zone of inhibition was measured and recorded (in mm). The activity tests were repeated on a separate day, using a fresh culture.

3.4.10.5 MIC determination

Minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial drug that will inhibit the visible growth of microorganism after overnight incubation. Minimum bactericidal concentration (MBC) is the lowest concentration of antibacterial agent required to kill the bacteria. Aqueous solutions of the benzalkonium-sulfacetamide along with the starting materials were prepared to give stock solution of 5000 µg/ml for benzyltriethylammonium chloride and benzyltriethylammonium sulfacetamide, 2000 µg/ml for benzyldimethylhexadecylammonium chloride, benzyldimethylhexadecylammonium sulfacetamide and sodium sulfacetamide, which was further diluted to design a set of concentrations from 2500 to 0.30 µg/ml and 1000 to 0.12 µg/ml in a nutrient broth (TSB). These different concentrations were used to investigate the synergistic antibacterial effect of benzalkonium ILs by broth dilution method. 200 µl of each sample were placed in a single well of three different 96-well plate (first row) – one plate per bacterial strain. 100 µl TSB was placed in each subsequent well of the plates; 0.1 ml of the sample from first row was transferred to row 2 and mixed thoroughly with the TSB by repetitive pipetting and then 0.1 ml of this was transferred in the same manner to subsequent rows such that a dilution series was set up. 100 µl

of TSB broth containing bacteria was transferred to all wells except the negative control column. After overnight growth, the absorbance of the wells was read using plate reader set to measure at 570 nm. The percentage bacterial growth inhibition was calculated by following formula.

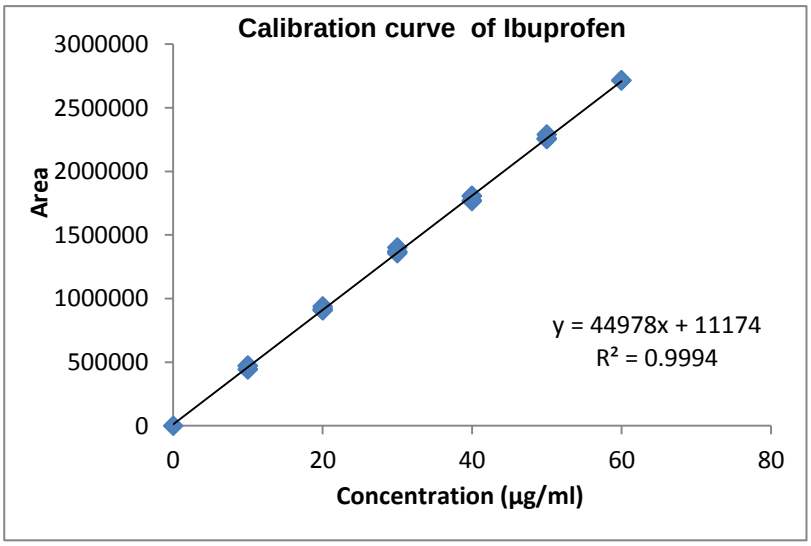
Percentage growth inhibition = (OD of control/ OD of control – OD of test sample) × 100

3.4.11 HPLC Analysis

The experiments were performed on Waters e2695 separation module with Waters 2998 photodiode array (PDA) detector. The chromatographic and the integrated data were recorded using Empower 3 software. Chromatographic separations were performed on Sunfire C18 5.0 µm (3.0 × 250 mm) column at 35 ºC using phosphate buffer pH 3.25 (A) and acetonitrile (B) as mobile phase with gradient elution at a flow rate of 1.1 mL/min. Gradient programme is shown in Table. 3.6. Detection of Ibuprofen and salicylic acid was carried at 191 nm and 202 nm respectively with adequate sensitivity. Calibration curve of ibuprofen and salicylic acid can be seen in Figure 3.8.

Table 3.5 Gradient programme for HPLC

	Time (min)	Flow (mL/min)	% A	% B
1		1.10	80.00	20.00
2	3.00	1.10	80.00	20.00
3	16.00	1.10	45.00	55.00
4	17.00	1.10	25.00	75.00
5	20.00	1.10	80.00	20.00
6	25.00	1.10	80.00	20.00



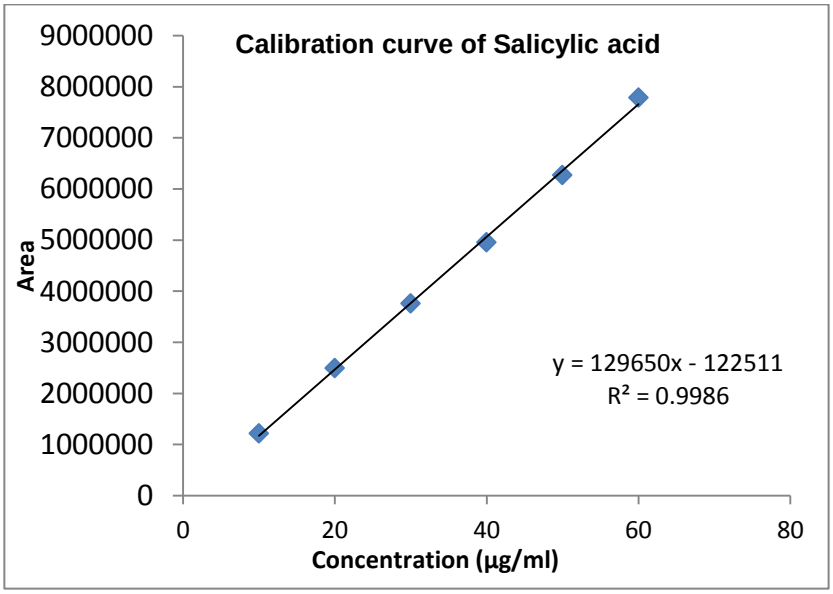


Figure 3.8 Calibration curve of ibuprofen and salicylic acid

Table 3.7 HPLC parameters for Ex-vivo studies

<table>
<tr><td colspan="4">HPLC analysis : Ex-vivo studies (collaborative work)</td></tr>
<tr><td>Sample</td><td>HPLC method parameters</td><td>Calibration equation</td><td>Instrument</td></tr>
<tr><td>[BTEA][Ibu]</td><td rowspan="2">Mobile phase
Methanol:water:acetic acid
600:377:23
Injection volume :10 µl
Detection wavelength : 264 nm
C18 column
Retention time: 7.85 min
Column tem: 35ºC</td><td rowspan="2">Y=844.18x
R^2=0.9998</td><td rowspan="5">Waters 2695 separations module (Model code SHC) with dual wavelength absorbance detector waters 2487</td></tr>
<tr><td>[BDMA][Ibu]</td></tr>
<tr><td>[BTEA][Diclo]</td><td rowspan="2">Mobile phase:
Methanol:Phosphate buffer pH 2.5
70:30
Injection volume: 10 ul
Detection wavelength:275nm
C18 column
Flow rate: 1ml/min
Retention time: 5.4 min
Column temp: 35ºC</td><td rowspan="2">Y=22881x
R^2=1</td></tr>
<tr><td>[BDMA][Diclo]</td></tr>
<tr><td>[BTEA][Sulfa]</td><td rowspan="2">Mobile phase
methanol:water:acetic acid
10:89:1
Injection volume: 10 ul
Detection wavelength:254nm
C18 column
Flow rate: 1ml/min
Retention time: 6.6 min
Column temp: 35ºC</td><td rowspan="2">Y=27372x
R^2=0.9999</td></tr>
<tr><td>[BDMA][Sulfa]</td></tr>
</table>

Table 3.8 HPLC parameters for in vitro permeation and release studies

<table>
<tr><td colspan="4">HPLC analysis : In vitro permeation and release studies</td></tr>
<tr><td>Sample</td><td>HPLC method parameters</td><td>Calibration equation</td><td>Instrument</td></tr>
<tr><td>M1
M2
M3
M4</td><td>Method: Gradient
Mobile phase
Acetonitrile : Phosphate buffer pH 3.25
Injection volume: 50 ul
Detection wavelength : 191 nm (Ibuprofen) and 202 (Salicylic acid)
C18 column
Column tem: 35ºC
Flow rate: 1.10 ml/min
Retention time: 4.57 min (salicylic acid) and 18.08 min (Ibuprofen)</td><td>y = 44978x + 11174
R² = 0.9994
(Ibuprofen)

y = 129650x - 122511
R² = 0.9986
(Salicylic acid)</td><td rowspan="2">waters e2695 separation module with waters 2998 photodiode array (PDA) detector</td></tr>
<tr><td>S1
S2
S3
S4
S5
S6
S7</td><td>Method: Gradient
Mobile phase
Acetonitrile : Phosphate buffer pH 3.25
Injection volume: 50 ul
Detection wavelength : 191 nm
C18 column
Column tem: 35ºC
Flow rate: 1.10 ml/min
Retention time: 18.08 min</td><td>y = 44978x + 11174
R² = 0.9994</td></tr>
</table>

Chapter 4

Benzalkonium based NSAIDs Ionic liquids (ILs)

4.1. Introduction

The focus of this chapter is investigation of the effect of counterions on the physicochemical properties and biopharmaceutical performance of benzalkonium based NSAIDs ILs for topical treatment. For this, sodium salt of Ibuprofen and sodium salt of diclofenac has been selected as a model drug in the form of anion and benzalkonium halides as cationic form which was further transformed into ILs by metathesis reaction and were characterised by NMR and IR spectroscopy. DSC, TGA, electrical conductivity, octanol-water partition coefficient measurements and *ex-vivo* skin deposition behaviour was also evaluated.

As discussed in Chapter 1, topical and transdermal drug delivery offers several advantages over oral drug delivery especially in case of non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and diclofenac. The most prevailing disease in the world is rheumatoid arthritis and its related disease. Diclofenac is non-steroidal anti-inflammatory drug (NSAID) which displays poor aqueous solubility because of its high melting point and intrinsic hydrophobicity. The carboxylic group is the only hydrophilic functional group which is also not available to interact with the solvent due to formation of dimer (Cordero et al. 1997). Stratum corneum is the outermost layer of the skin which functions as hydrophobic barrier for absorption of most of the drugs.

During the 19th century, the antibacterial properties of quaternary ammonium compounds were discovered among malachite green, methyl violet and auramin (Dunn 1949; Hough-Troutman et al. 2009). The complete potential of

quaternary ammonium compounds was acknowledged after the synthesis of benzalkonium chloride in 1935, which was found to possess a wide spectrum of effectiveness against many bacterial strains after characterization of its anti-bacterial properties (Domagk 1935). These newly emerged water soluble quaternary ammonium compounds display anti-bacterial properties against not only gram-positive and gram-negative bacteria, but also against protozoa and pathogen species of fungi (Kull et al. 1961). Interestingly, these compounds can also act as penetration enhancers for transbuccal and transnasal drug delivery systems such as nasal vaccinations (Klinguer et al. 2001). Later these water soluble quaternary ammonium compounds have shown potential applications in the fields of disinfectants (Lopes 1986), surfactants (Hayakawa and Kwak 1991), phase transfer catalysis (Makosza 2000) and anti-corrosive agents (Hough-Troutman et al. 2009). Certainly a surfactant molecule is useful to modify the texture of formulation and also helps to stabilize it. In addition, surfactants can alter the transport across biological barriers by directly affecting the biological membrane (Khossravi 1997; Savić et al. 2010). The antibacterial mechanism of action of quaternary ammonium compounds is based on the disruption of membrane charge distribution and altering cell membrane permeability, leading to leakage of all components from cytoplasm, followed by bacterial death (Arias-Moliz et al. 2015).Utilizing API-IL approach not only lowers the melting point of the API but also helps to generate room temperature IL salts.

In light of the above, we investigated the feasibility of topical application of benzalkonium based NSAIDs ILs and evaluated the impact of counterions on the dermal bioavailability and the physicochemical properties. It is worthy to note that there is no reported data in the literature on the skin deposition and

permeation profiles of these anions (ibuprofen, diclofenac) combined with benzalkonium.

4.2. Results and discussions

4.2.1 Anion exchange reaction of benzalkonium based NSAIDs ILs and characterisation

All synthesized benzalkonium based NSAIDs ILs were in the form of clear yellow viscous liquids to colourless viscous liquids at room temperature (Table 4.1).

The benzalkonium based NSAIDs ILs were characterized by ^{1}H NMR, ^{13}C NMR and FTIR spectroscopy (Figure 4.1 – 4.8). The proton signals for [BTEA] were recorded at 1.25- 7.47 ppm and included a signal for 9 protons of its terminal methyl groups at 1.25 ppm (t), 6 protons of adjacent methylene groups were found at 3.09-3.21ppm (q), 2 protons for the methylene next to phenyl group observed at 4.45 ppm (s) while the aromatic protons were found at 7.35-7.47 (m). The signals obtained for [BTEA] were compared with the signals of respective sodium salts of APIs confirming the formation of ILs, indicating good stability of active pharmaceutical ingredient during salt formation. This data was further supported by the IR spectroscopy. The formation of ILs takes place through anion exchange reaction using sodium salt of APIs. Therefore the typical C=O stretching vibrations between 1690 cm^{-1} – 1709 cm^{-1} exhibited by carboxylic group containing APIs were not important to this study. Following the metathesis reaction the symmetric and antisymmetric stretching of carboxylic anions were found at 1583 cm^{-1} and 1378 cm^{-1} [BTEA-Ibu], 1584 cm^{-1}, 1363 cm^{-1} [BTEA-Diclo], 1581 cm^{-1} and 1370 cm^{-1} [BDMA-Ibu] and 1585 cm^{-1}, 1363 cm^{-1} [BDMA-Diclo] respectively.

NMR and FTIR characterization data

Benzyltriethylammonium-ibuprofen [BTEA] [Ibu]: Yield 78%.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.86 (d, 6 H), 1.25 (t, 9 H), 1.38 (d, 3 H), 1.77 (m, 1 H), 2.35 (d, 2 H), 3.09 - 3.21 (q, 6 H), 3.52 (q, 1 H), 4.45 (s, 2 H), 6.93 (m, 2 H), 7.29 (m, 2 H), 7.35 - 7.47 (m, 5 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 7.93, 18.40, 19.96, 22.34, 30.17, 44.99, 49.30, 52.37, 57.07, 60.59, 76.93, 77.25, 77.46, 77.57, 127.36, 127.44, 128.40, 129.22, 130.44, 132.31, 138.19, 143.06, 179.56

IR (v_{max} cm^{-1}): 3086, 2956, 2845, 2346, 1583, 1458, 1378, 1153, 1008, 778, 713, 617

Benzyltriethylammonium-Diclofenac [BTEA] [Diclo]: Yield 81%.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.13 - 1.26 (t, 9 H), 3.03 (q, 6 H), 3.64 (s, 2 H), 4.31 (s, 2 H), 6.36 (dd, 1 H), 6.62 - 6.74 (dt, 1 H), 6.79 - 6.91 (dt, 1 H), 7.14 (t, 1 H), 7.24 (d, 2 H), 7.28 - 7.39 (m, 5 H), 9.79 (s, 1 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 7.93, 18.49, 44.66, 52.30, 57.67, 60.47, 76.79, 77.11, 77.31, 77.43, 116.53, 120.07, 120.15, 122.71, 125.68, 127.20, 128.75, 129.15, 129.30, 130.37, 130.51, 132.27, 138.67, 143.55, 177.12, 177.16

IR (v_{max} cm^{-1}): 3208, 2968, 2323, 1606, 1584, 1451, 1363, 1049, 784.4, 703.3, 643.7

Benzyldimethylhexadecylammonium-ibuprofen [BDMA] [Ibu]: Yield 76%.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.85 (d, 6 H), 0.86 (t, 3 H), 1.20 - 1.32 (m, 26 H), 1.45 (d, 3 H), 1.54 - 1.67 (m, 2 H), 1.76 (m, 1 H), 2.33 (d, 2 H), 3.05 (s, 6 H), 3.17 - 3.28 (m, 2 H), 3.53 - 3.65 (q, 1 H), 4.74 (s, 2 H), 6.93 (d, 2 H), 7.30 - 7.42 (m, 5 H), 7.43 - 7.48 (m, 2 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 14.12, 19.87, 22.44, 22.69, 22.80, 26.32, 29.28, 29.37, 29.40, 29.49, 29.61, 29.66, 29.70, 30.23, 31.93, 45.11, 49.52, 63.20, 67.29, 76.73, 77.05, 77.26, 77.37, 127.52, 127.75, 128.52, 129.05, 130.39, 133.19, 138.28, 143.19, 179.61

IR (v_{max} cm^{-1}): 3017, 2955, 2921, 1581, 1462, 1370, 1345, 864.8, 782.7, 734.9, 708.2

Benzyldimethylhexadecylammonium-Diclofenac [BDMA][Diclo]: Yield 76%.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.81 - 0.93 (t, 3 H), 1.23 - 1.35 (m, 26 H), 2.93 (s, 6 H), 3.58 - 3.69 (s, 2 H), 4.57 (s, 2 H), 6.41 (dd, 1 H), 6.66 - 6.77 (dt, 1 H), 6.81 - 6.93 (dt, 1H), 7.18 (dd,1 H), 7.25 (d, 2 H), 7.29 - 7.42 (m, 5 H), 9.63 (s, 1 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 14.13, 18.48, 22.70, 22.81, 26.30, 29.27, 29.37, 29.46, 29.59, 29.64, 29.68, 29.70, 31.93, 49.55, 58.23, 76.72, 77.04, 77.36, 116.70, 120.22, 122.69, 125.75, 127.55, 128.76, 129.05, 129.21, 129.42, 130.43, 133.16, 143.53, 177.31

IR (v_{max} cm^{-1}): 3032, 2923, 2853, 1606, 1585, 1452, 1363, 1050, 866.8, 744.2, 642

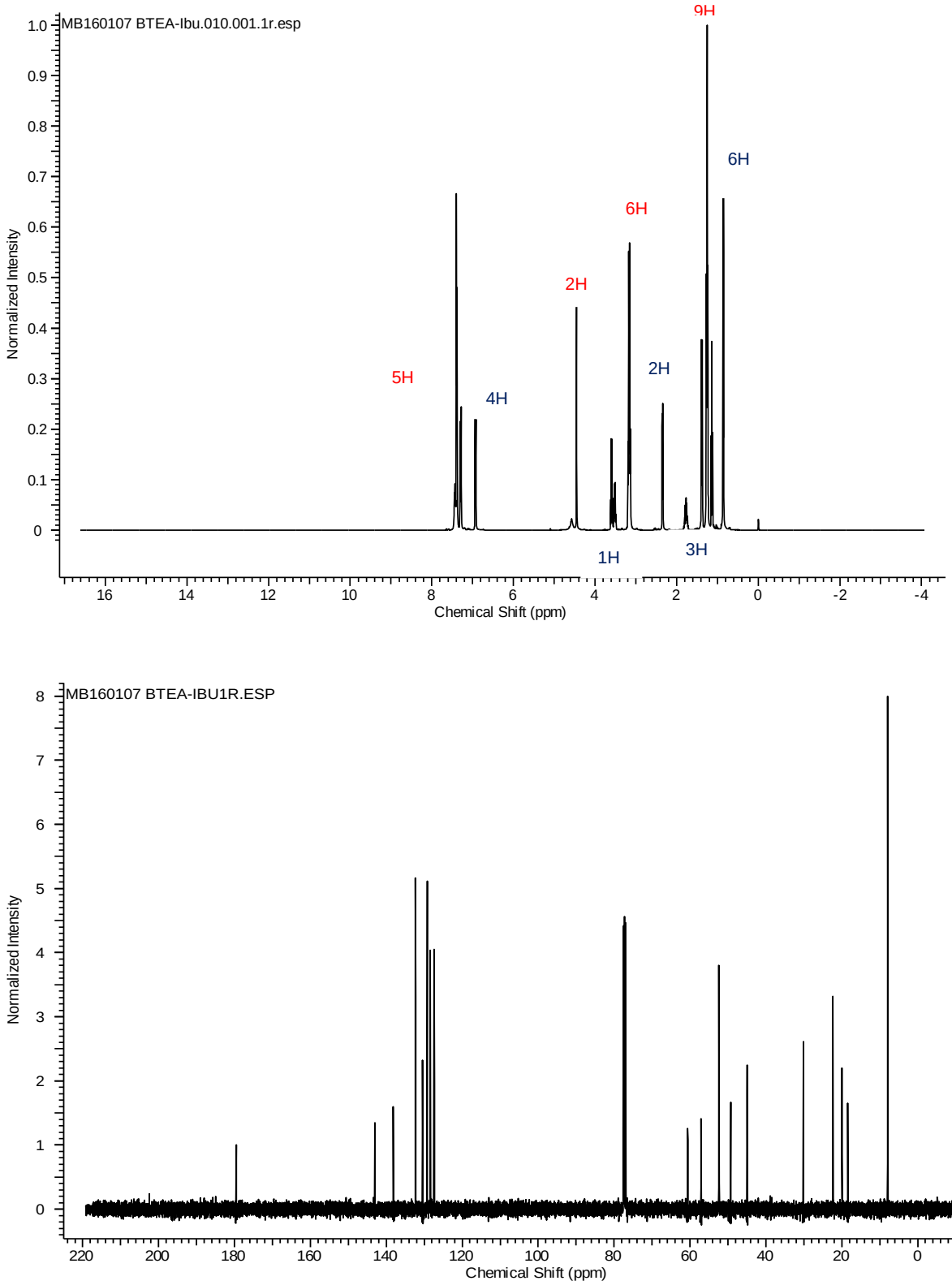


Figure 4.1 ^{1}H NMR and ^{13}C spectra of [BTEA] [Ibu]

[BTEA] and [Ibu] protons are represented in red and blue colours respectively

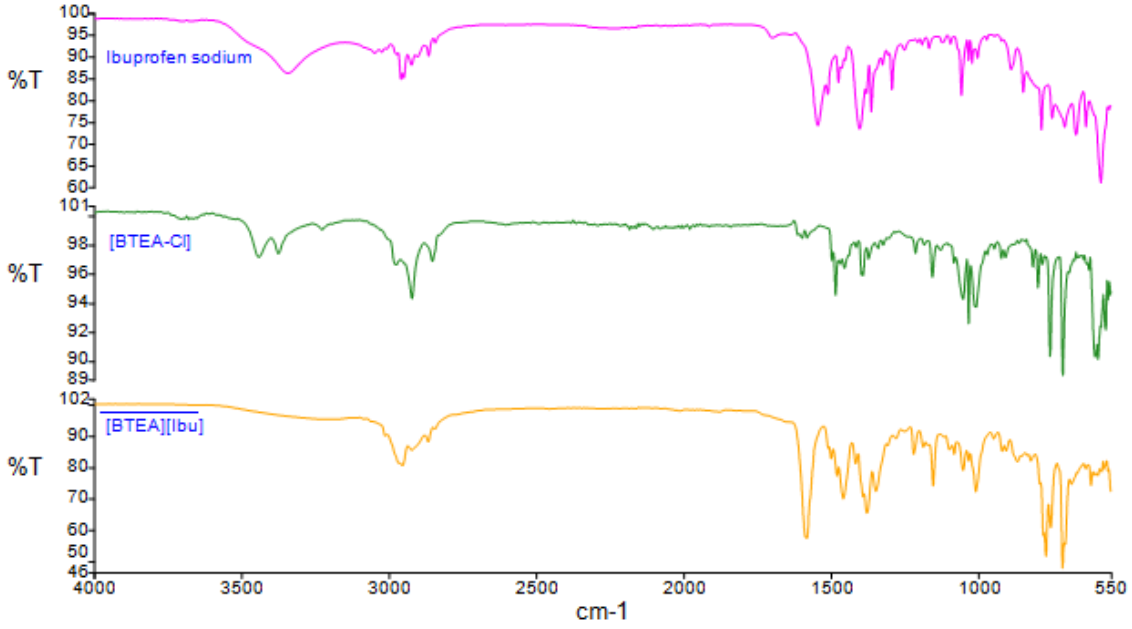


Figure 4.2 FTIR spectra of [BTEA] [Ibu]

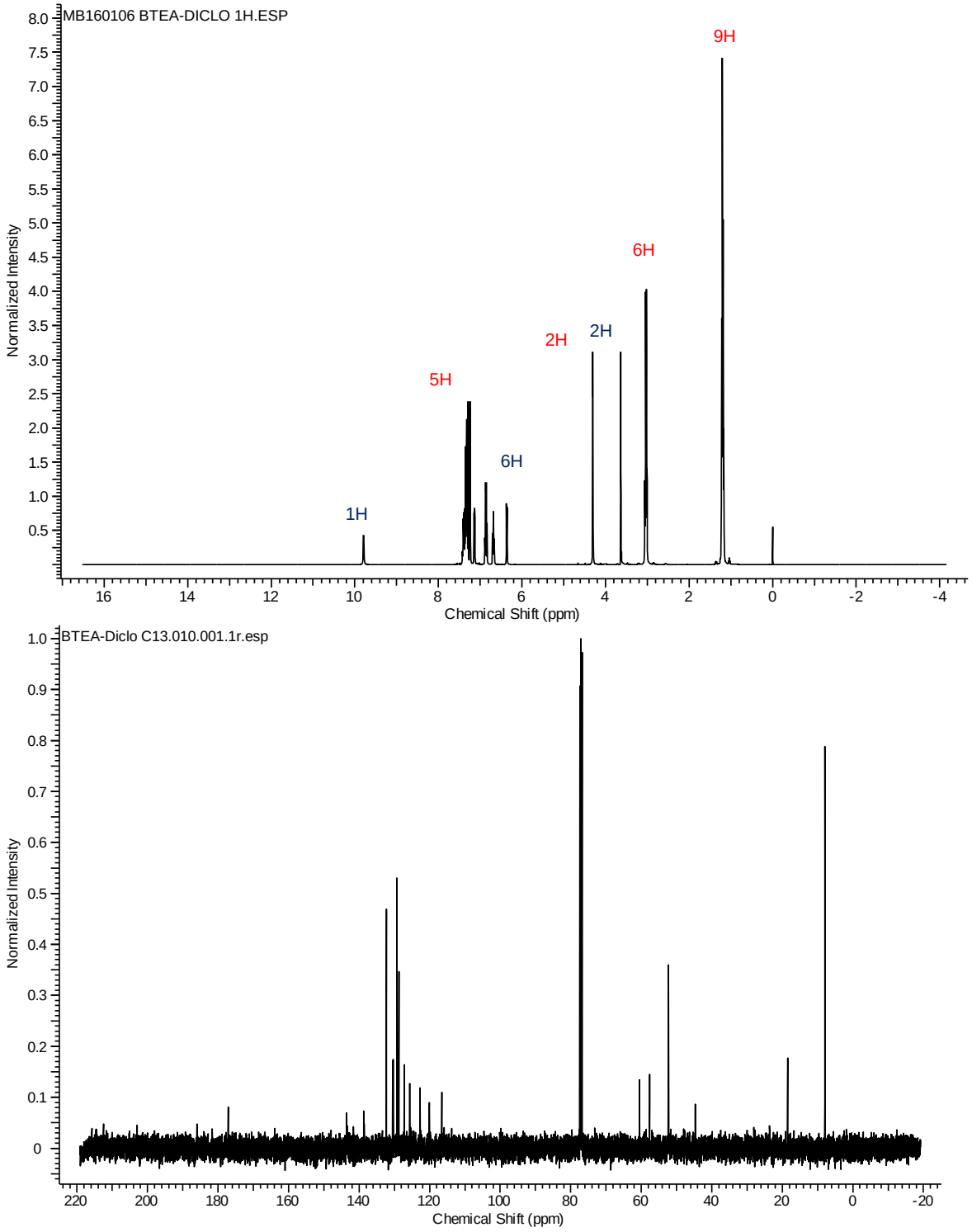


Figure 4.3 ^{1}H NMR and ^{13}C spectra of [BTEA] [Diclo]

[BTEA] and [Diclo] protons are represented in red and blue colours respectively

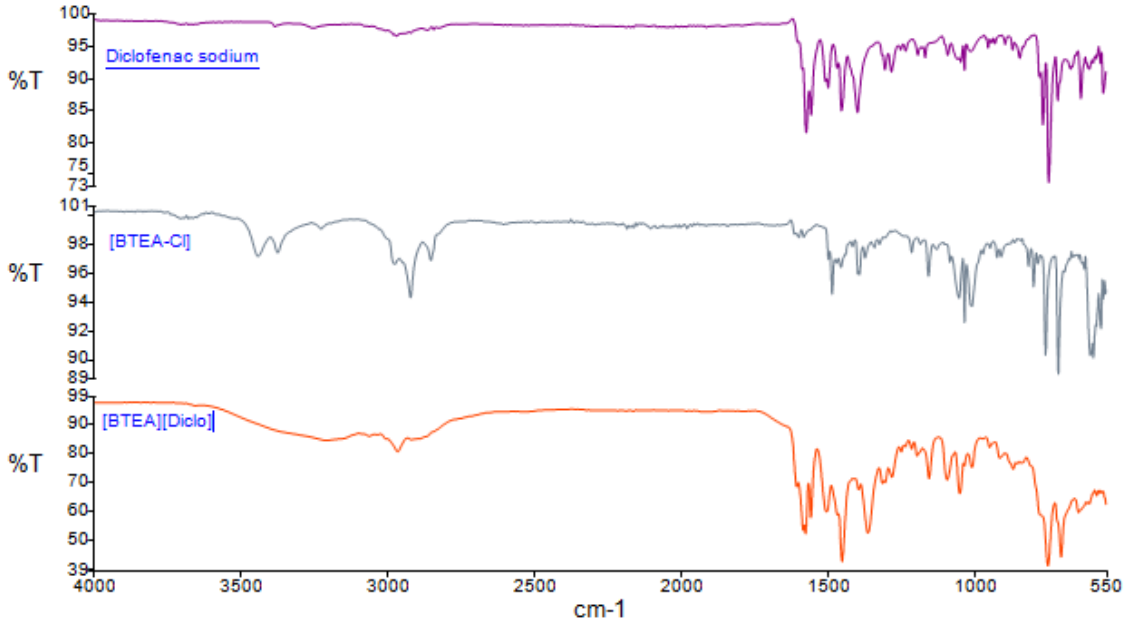


Figure 4.4 FTIR spectra of [BTEA] [Diclo]

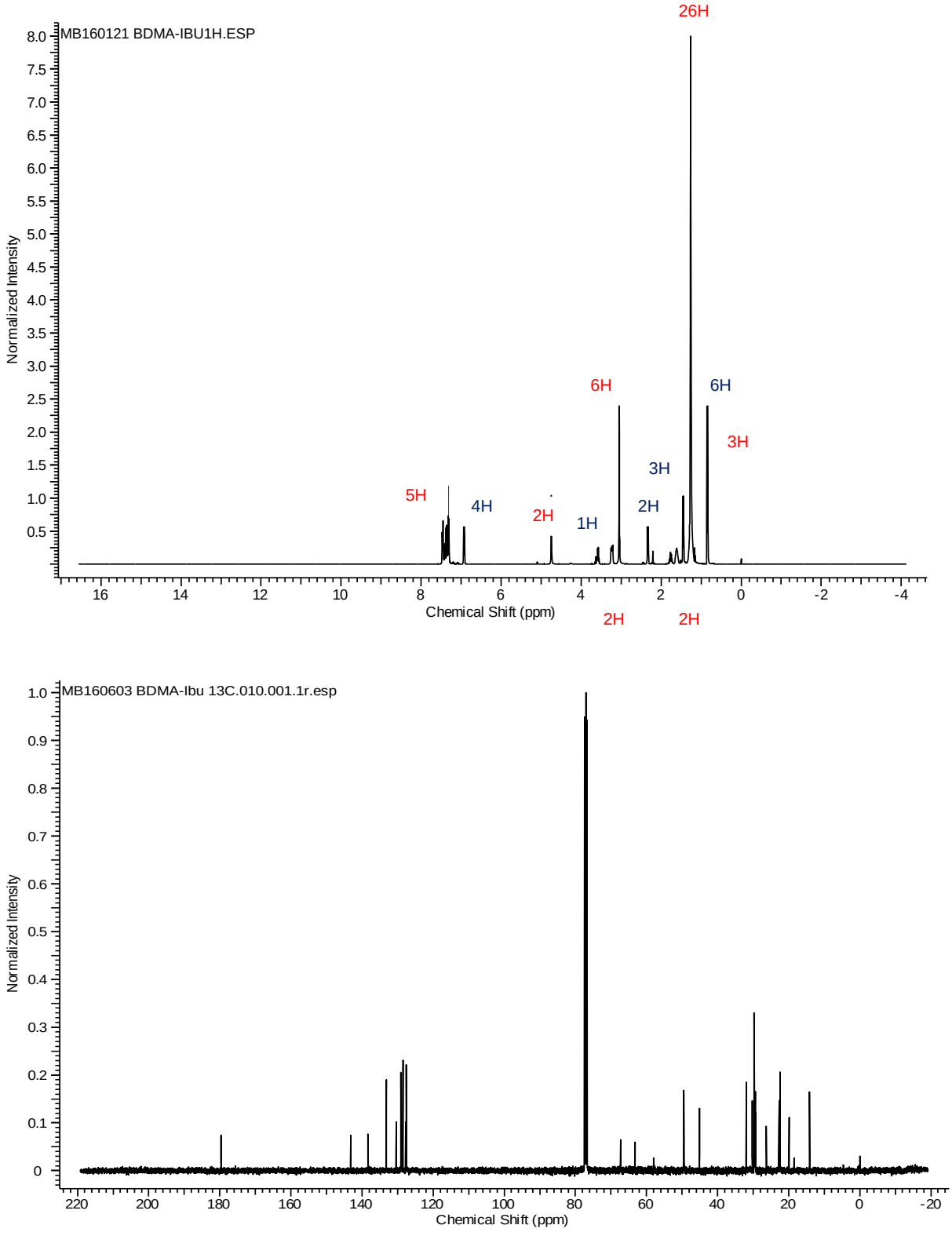


Figure 4.5 ^{1}H NMR and ^{13}C spectra of [BDMA] [Ibu]

[BDMA] and [Ibu] protons are represented in red and blue colours respectively

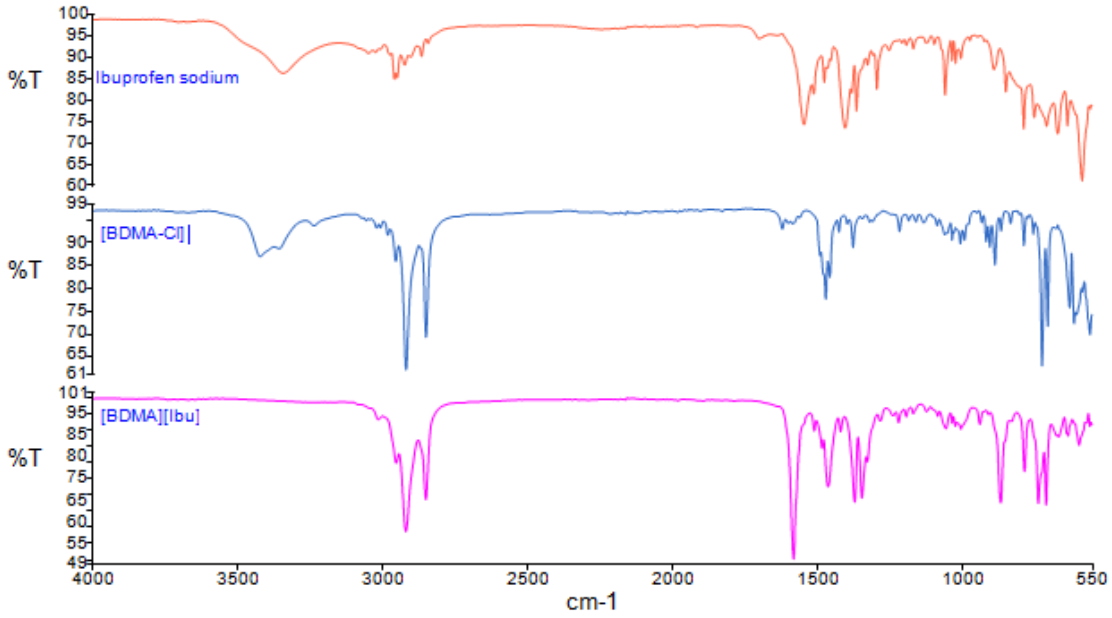


Figure 4.6 FTIR spectra of [BDMA] [Ibu]

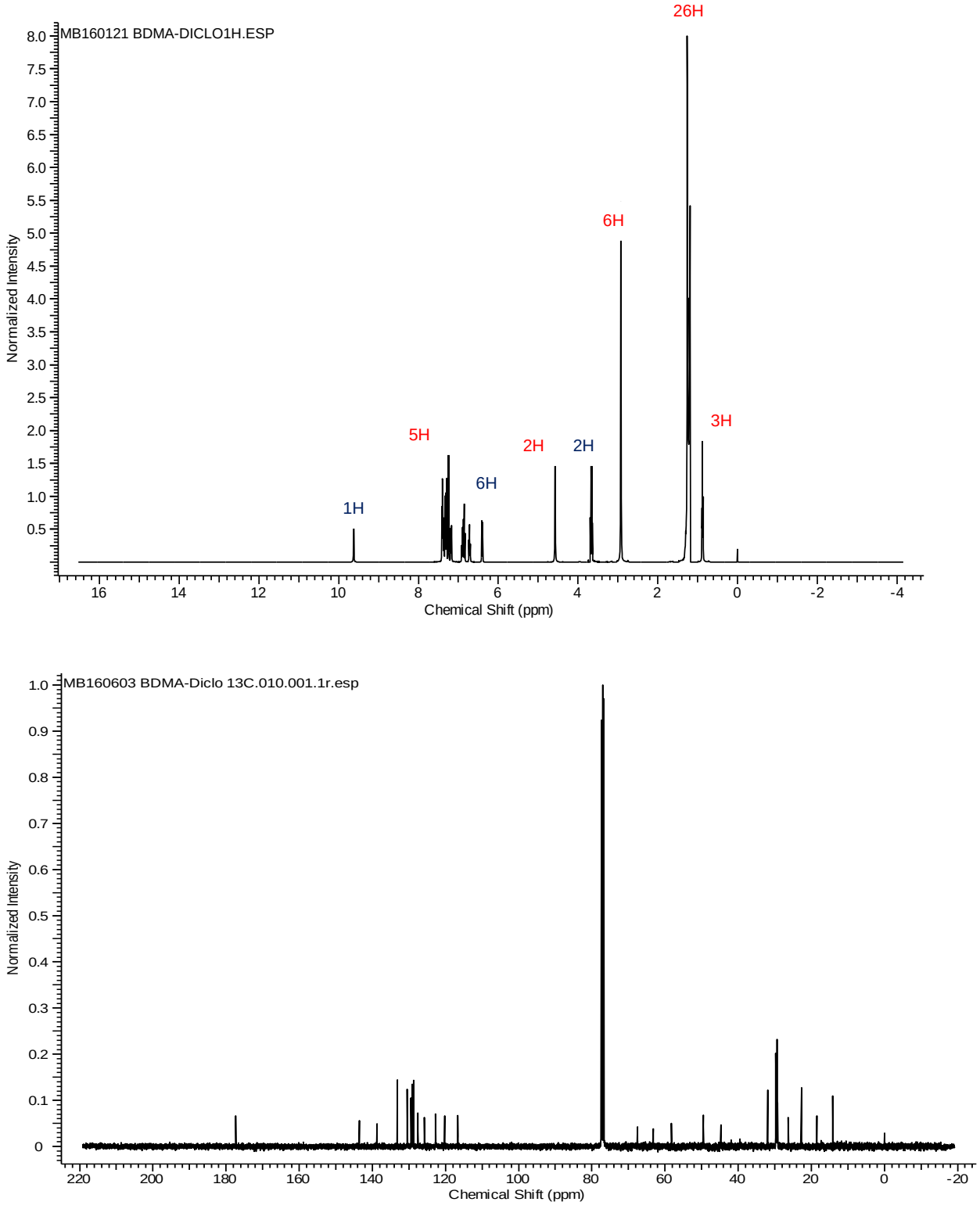


Figure 4.7^{1}H NMR and ^{13}C spectra of [BDMA] [Diclo]

[BDMA] and [Diclo] protons are represented in red and blue colours respectively

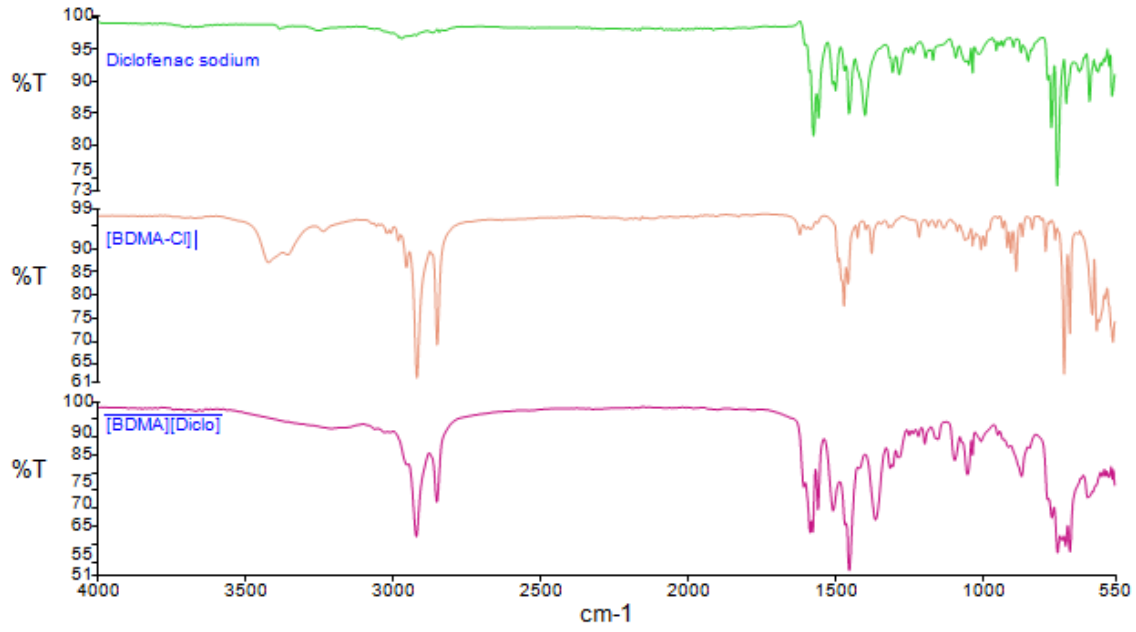


Figure 4.8 FTIR spectra [BDMA] [Diclo]

4.2.2 Thermal Behaviour

The effect of counterions on the thermal properties of the benzalkonium based NSAIDs ILs was studied using TGA and DSC (Figure 4.9 – 4.12). All the benzalkonium based NSAIDs ILs are viscous liquids at room temperature. Table.4.1 shows the thermal stability of studied benzalkonium based NSAIDs ILs ranging from 147.56 ˚C to 165.60 ˚C. Two step decomposition was observed for [BTEA] [Diclo], while the remainder of the studied benzalkonium based NSAIDS ILs exhibited one step decomposition process.

The thermal decomposition temperature of [BTEA] based NSAIDs ILs were found in the order of [BTEA] [Ibu] > [BTEA] [Diclo]. The impact of anions can be observed by the difference in thermal stability of API-ILs. It could be observed from the trend that thermal stability of the [BTEA] based NSAIDs ILs decreases with increase in anion size (asymmety), the negative charge is spread over the large volume of anion resulting in much weaker interactions between the counterions (Leys et al. 2008). The same pattern was observed for [BDMA] based NSAIDs ILs, [BDMA] [Ibu] > [BDMA] [Dic]. However, when you compare the decomposition temeratures of [BTEA] and [BDMA] based NSAIDs ILs then it was observed that [BDMA] based NSAIDs ILs display higher decomposition temperatures. The increase in decomposition temperature could be attributed due to increase in interparticle forces (Van der Waal forces) which arises with increase in alkyl chain length (Canongia Lopes and Padua 2006; Chancelier et al. 2016).

DSC thermograms showed that the glass transition temperature for all the synthesized benzalkonium based NSAIDs ILs were in the range of -39.10 to -37.95 for [BTEA] based NSAIDs ILs and -33.54 to -7.35 for [BDMA] based NSAIDs ILs respectively (Table 4.1). The glass transition temperature of [BTEA]

based NSAIDs ILs were found in the order of [BTEA] [Diclo] > [BTEA] [Ibu]. The observed trend could be correlated to the molecular volume. Higher the molecular volume lowers the coulombic force of attraction between ions leading to increase in glass transition temperature values. Similar trend was observed for [BDMA] based NSAIDs ILs, [BDMA] [Diclo] > [BDMA] [Ibu].Regardless of being in liquid phase at room temperature, the [BTEA] and [BDMA] based NSAIDs ILs displayed different thermal response, in which [BDMA] based NSAIDs ILs exhibited higher glass transition temperature compared to [BTEA] based NSAIDs ILs which could be correlated to the increase in molar volumes leading to increase in Van der Waals force of attraction (Leys et al. 2008).

Table 4.1 Thermal properties of benzalkonium based NSAIDs ILs

S.no	NSAIDs-ILs	Tg(°C)	Td(°C)	Physical state
1	[BTEA] [Ibu]	-39.10	147.56	Colourless Viscous liquid
2	[BTEA] [Dic]	-37.95	141.85	Yellow viscous liquid
3	[BDMA] [Ibu]	-33.54	165.60	Colourless Viscous liquid
4	[BDMA] [Dic]	-7.35	157.02	Yellow viscous liquid

Tg – glass transition temperature ; Td – decopmposition temperature

TGA and DSC profiles of benzalkonium based NSAIDs ILs

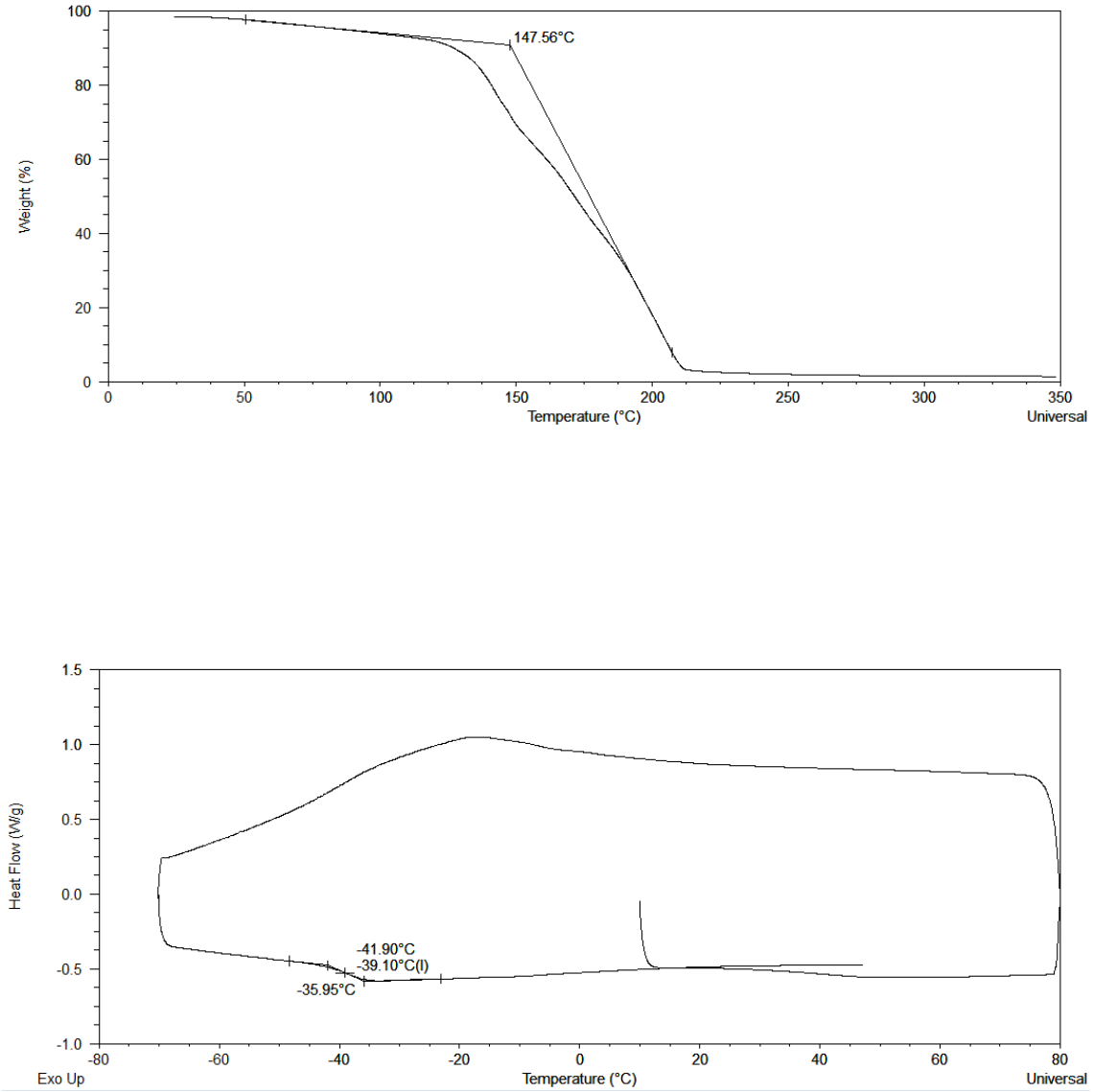
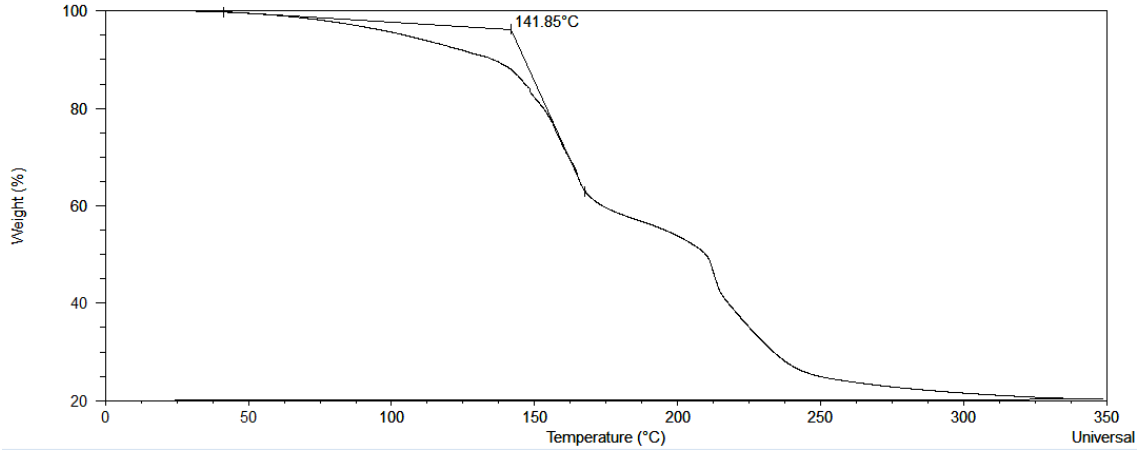


Figure 4.9 TGA and DSC profile of [BTEA] [Ibu]



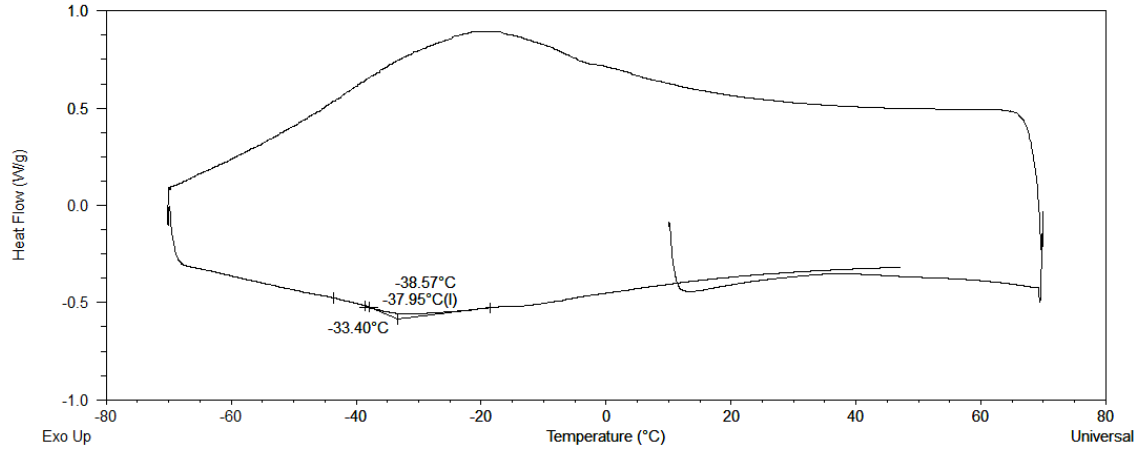


Figure 4.10 TGA and DSC profile of [BTEA] [Diclo]

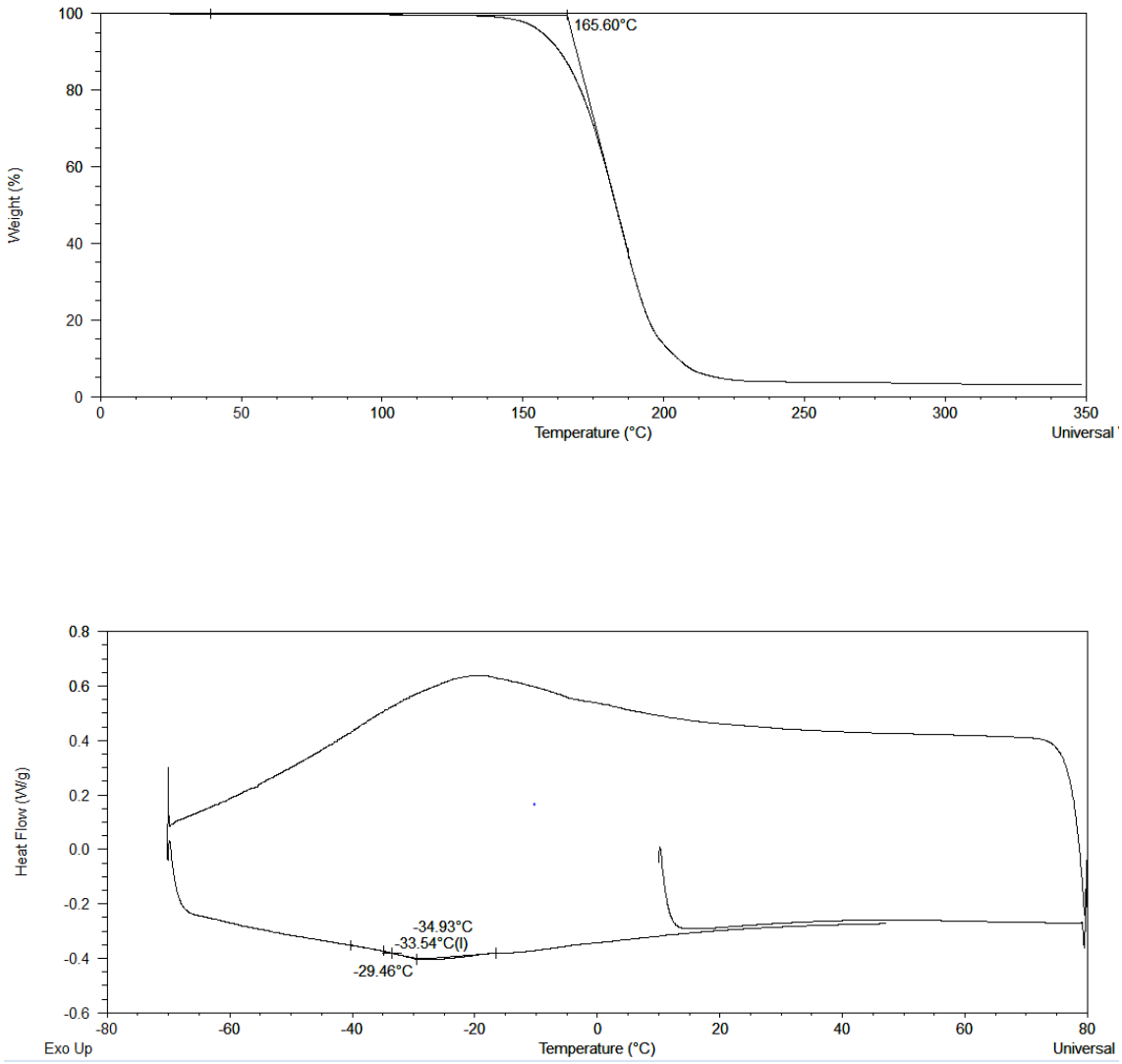
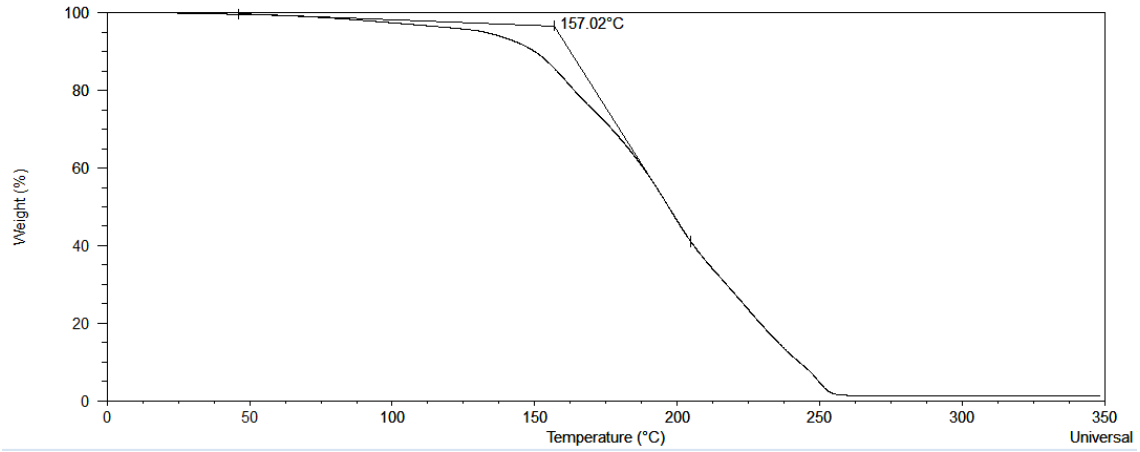


Figure 4.11 TGA and DSC profile of profile of [BDMA] [Ibu]



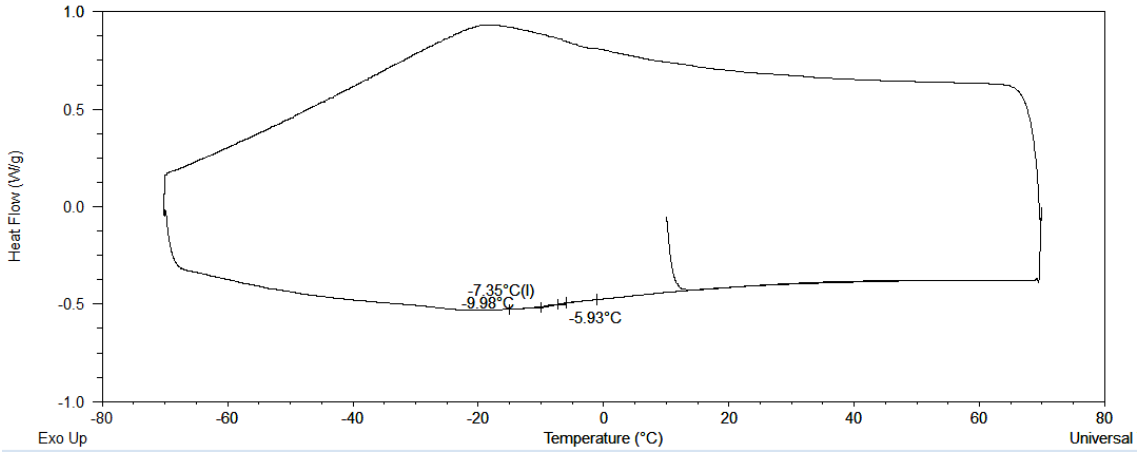


Figure 4.12 TGA and DSC profile of profile of [BDMA] [Diclo]

4.2.3 Electrical Conductivity studies

The ability of a material to accommodate transport of electric charge is referred to as its electrical conductivity. A large conductivity was expected for ILs because they are entirely composed of charge carrier ions. The electrical conductivity of ILs is different from solutions having dissociated ions or nonpolar liquids. This indicated that measurement of electrical conductivity of synthesized ILs at different concentrations in water would be of interest (Widegren et al. 2005).

In order to investigate the influence of counterions on the conductivity, the electrical conductivity profiles of aqueous solutions of [BTEA] based NSAIDs ILs were studied at high concentration range, and the concentration range for all [BTEA] based NSAIDs ILs was kept constant. The conductivity of [BTEA] based NSAIDs ILs is shown in Figure. 4.13. The room temperature conductivity values of [BTEA] [Ibu] were found to be higher compared to [BTEA] [Diclo]. The decrease in conductivity values correlated with increasing anion size, which led to reduction of effective anion charge density because the negative charge is spread over the much large volume. Therefore, the interaction of these anions with benzalkonium cation becomes much weaker (Leys et al. 2008). In addition to this, [BTEA] [Ibu], [BTEA] [Diclo] showed different behaviour than that of the salt. The dissociation of ILs into free ions transporting electrical charge was observed at low molar concentration; up to a certain concentration, the conductivity increases with the increasing salt concentration and reaches to a maximum then gradually falls with further increase in salt concentration. At high concentration ILs possessed low electrical conductivity due to reduced ionic

mobility attributed to increased viscosity, polarization effect at interface of electrode, large constituent ions of ILs and cation-anion interactions. The ions contributing to ILs have different degrees of cation-anion interactions at intermediate concentrations (Park and Prausnitz 2015). Two different regions exist in the conductivity profiles of ionic solutions. In the first region the conductivity increases because ionic charges could be considered as high mobility charge carriers while in the second region the conductivity was found to be lower as a result of low mobility of charge carrying ions due to increased ionic interaction effects (Goindi et al. 2015)

As described earlier, to investigate the conductivity profiles of [BDMA] based NSAIDs ILs, the aqueous solutions were prepared at high concentration. [BDMA] [Ibu] drug salt successfully managed to form aqueous solution; but at low concentration of the drug salts, while [BDMA] [Diclo] failed to give clear solutions due to hydrophobic nature and viscosity. The electrical conductivity value for [BDMA] [Ibu] is represented in Figure. 4.13. It can be observed from the figure that electrical conductivity data followed the trend of the salt in aqueous solution, where the conductivity increases with increase in concentration of ILs. Aqueous solution of ILs behaves similar way to concentrated salt solutions. The conductivity of ILs aqueous solution increases due to the presence of water-rich regions. The dissociation constant of ILs was increased with increase in the amount of water (Tshibangu et al. 2011).

However, comparing the electrical conductivity results of [BTEA] and [BDMA] based NSAIDs ILs shows that [BDMA] based NSAIDs ILs displayed a decrease in electrical conductivity with increase in the alkyl chain length. Since the conductivity of the system is due to mobility of the charge carriers, the increase in the volume fraction of the hydrocarbon portion of the cation increases the

molecular volume of the organic cation which leads to induce strong Van der Waals interactions. These attractive forces induce higher viscosity which results in decrease in electrical conductivity (Leys et al. 2008; Papović et al. 2016).

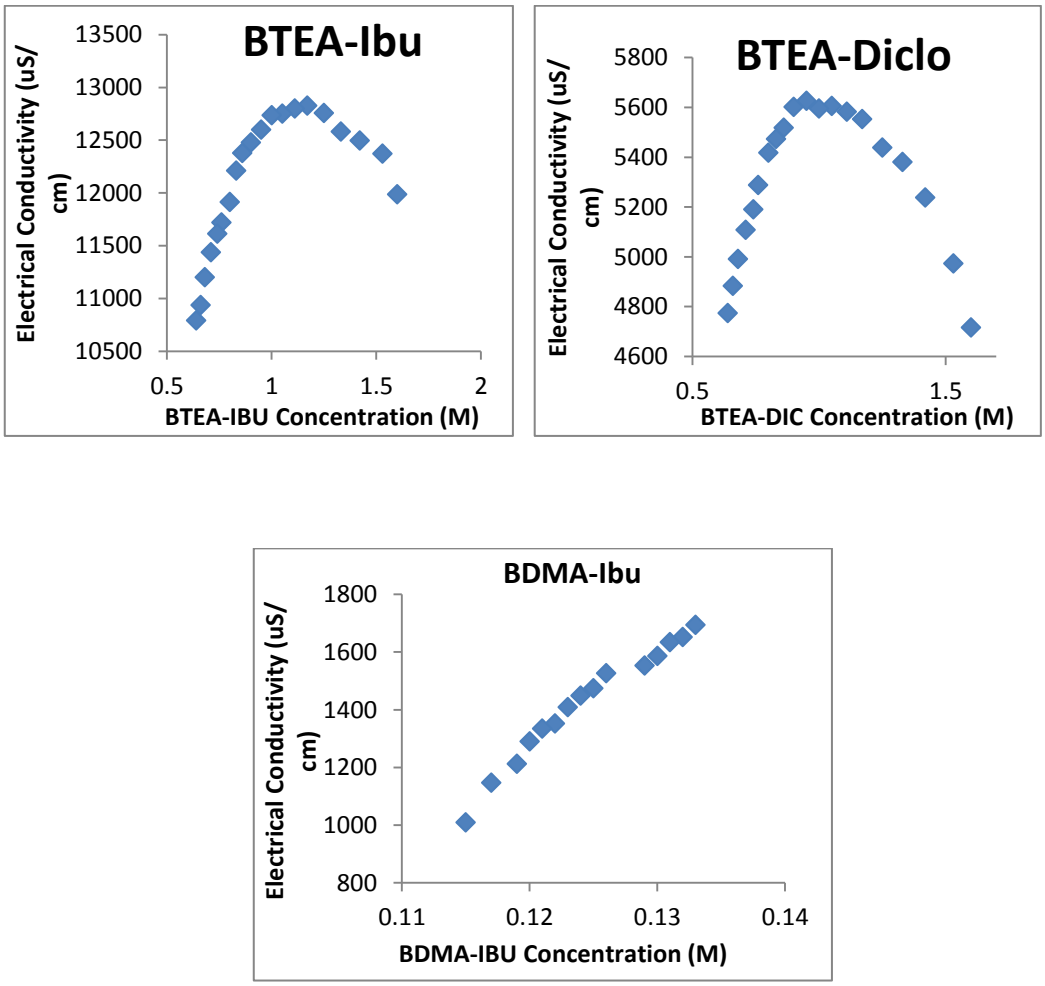


Figure 4.13 Electrical conductivity of aqueous solutions of [BTEA] and [BDMA] based NSAIDs ILs

4.2.4 Octanol-water partition coefficient

The lipophilicity of a chemical compound is estimated by its octanol/water partition coefficient and is a key factor in the formulation of pharmaceuticals. It is a significantly important property of the molecule which gives an idea of its affinity towards hydrophilic and hydrophobic environments, and has been correlated with adsorption and transport properties of drug molecules in the

human body as well as sorption to sediments and soils. A number of factors are responsible for governing the value of octanol-water partition coefficient including concentration, pH, molecular weight, molecular volume (Ingram et al. 2011).

The octanol-water partition coefficient of benzalkonium based NSAIDs ILs was studied to understand the passive diffusion across biological membranes. The results as shown in Table 4.2 indicate that the [BTEA] based NSAIDs ILs are more hydrophilic than [BDMA] based NSAIDs ILs. In particular, the octanol-water partition coefficient ($K_{o/w}$) values are higher for [BDMA] based NSAIDs ILs than [BTEA] based NSAIDs ILs which could be attributed to increase in molecular weight and alkyl chain length on the cation as shown in Figure.4.14(Domańska et al. 2003; Wu et al. 2003; Crosthwaite et al. 2004).

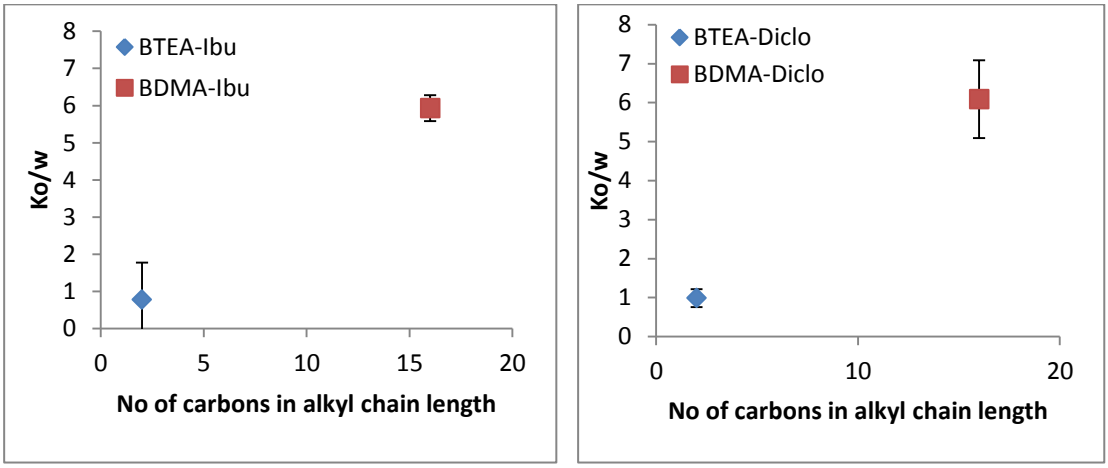


Figure 4.14 Relationship between the number of carbon in the alkyl chain length on the cation and Ko/w profiles (Error bars - standard deviation)

The significant effect of different anions on the octanol-water partition coefficient values can be determined by keeping the cation constant (Figure.4.15). The octanol-water partition coefficient values of [BTEA] based NSAIDs ILs displayed

the following trend: [BTEA] [Diclo] > [BTEA] [Ibu].[BTEA] based NSAIDs ILs $K_{o/w}$ values were found to be less than 1 as they are totally soluble in water. All the drug salts are water miscible at room temperature and followed the trend of anion hydrophobicity (Leo et al. 1971; Fini et al. 1999; Crosthwaite et al. 2004). The same pattern was observed for the [BDMA] based NSAIDs ILs. The $K_{o/w}$ value of [BDMA-Diclo] was found to be high compared to the rest of the studied API-ILs because of the hydrophobic nature of the drug and high molecular weight of the API-IL.

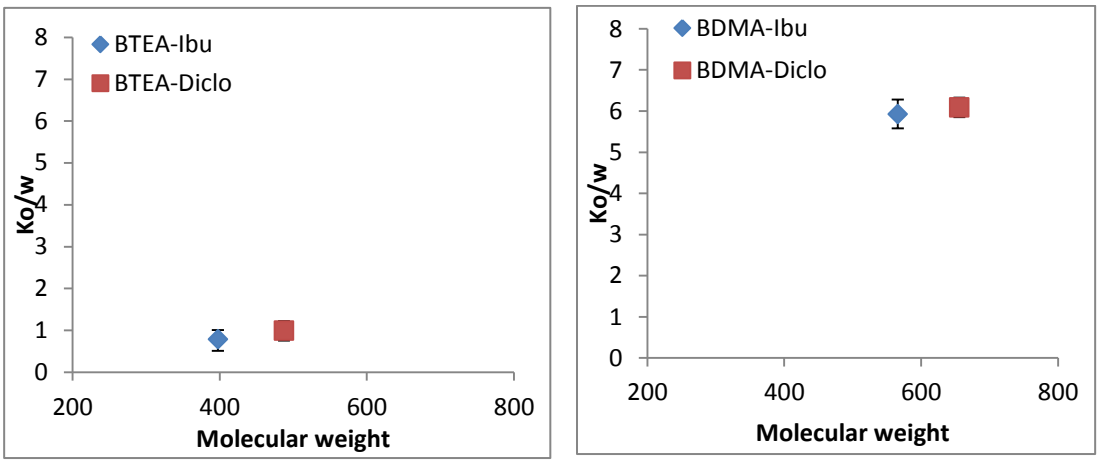


Figure 4.15 Effect of anions on Ko/w keeping cation constant (Error bars - standard deviation)

4.2.5 Ex-vivo skin studies

4.2.5.1 Drug deposition study

Ex-vivo permeation and deposition studies were performed to determine the rate and the extent of drug salts permeated and deposited in the various layers of skin.

In order to compare the skin deposition values of anionic APIs (ibuprofenate, diclofenac) in synthesised benzalkonium based NSAIDs ILs, the amount of drug retained in stratum corneum, epidermis and dermis was evaluated after the *ex-vivo* skin permeation experiment through rat skin. The skin deposition values of

anionic APIs (ibuprofenate, diclofenac) based on [BTEA] cation are displayed in Table 4.2 and Figure.4.16. The [BTEA] [Diclo] content was found in higher amount in stratum corneum and epidermis of rat skin, which could be attributed to the lipophilisation of diclofenac ion when combined with benzalkonium cation. Surprisingly, there was no significant difference observed in the [BTEA] [Diclo] and [BTEA] [Ibu] content in dermis. The flux values for [BTEA] [Ibu] was expected to be greater than [BTEA] [Diclo] on the basis of molecular volume but it seems that the impact of partition coefficient overrules the influence of molecular volume (Scheuplein et al. 1969).

Table 4.2 Skin deposition and permeation amounts ofbenzalkonium based NSAIDs ILs

NSAIDs-ILs	MW	Ko/w	Deposited % in stratum corneum	Deposited % in Epidermis	Deposited % in Dermis	% Drug permeated
[BTEA] [Ibu]	397.6	0.78 (±0.27)	2.48 (±0.05)	0.72(±0.14)	4.19(±2)	2.02% (±0.96)
[BTEA] [Diclo]	487.46	0.99(±0.23)	13.12(±1.28)	6.3(±0.75)	3.08(±0.42)	0.165% (±0.005)
[BDMA] [Ibu]	565.93	5.93 (±0.35)	23.39(±2.67)	13.87(±2.4)	10.87(±1.7)	0%
[BDMA] [Diclo]	655.79	6.09(±0.24)	32.61(±4.72)	9.54(±1.7)	8.39(±0.57)	0.037% (±0.007)

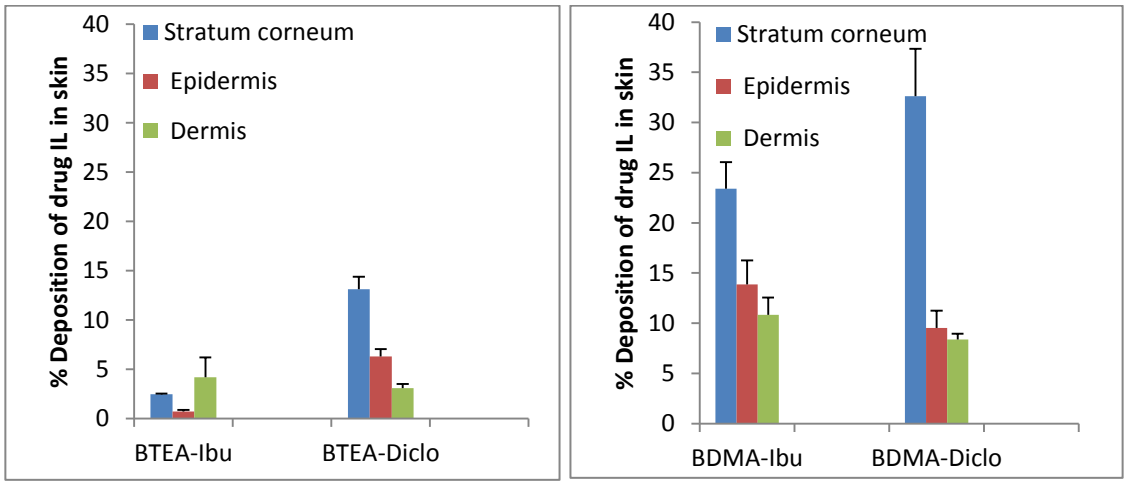


Figure 4.16Skin deposition profiles of [BTEA] and [BDMA] based NSAIDs ILs (Error bars - standard deviation)

In the case of [BDMA] based NSAIDs ILs, the skin deposition values of anionic APIs (ibuprofenate, diclofenac) are displayed in Table.4.2 and Figure.4.16. The [BDMA] [Diclo] content was found in higher amounts in stratum corneum of rat skin, which could be attributed to the increase in lipophilicity of the drug salt. This increases the solubility of drug salt within lipid domains of the stratum corneum and finally improves the membrane permeability (Flynn and Yalkowsky 1972). The effect of molecular weight on the drug flux seems to be relatively negligible compared to the influence of changes in partition coefficient (Scheuplein et al. 1969). On the other hand [BDMA] [Diclo] content was found to be the lower in epidermis and dermis. The most significant factor for reduced permeation across the membrane could be attributed to its hydrophobic nature and increase in the molecular volume of diclofenac anion molecule.

4.2.5.2 Drug permeation study

In order to compare the skin permeability of anionic APIs (ibuprofen, diclofenac) combined with benzalkonium cation,*ex vivo* permeation experiments were carried out through rat skin with the synthesised benzalkonium based NSAIDs ILs. Figure.4.17 shows the comparison of the cumulative permeation profiles of [BTEA] and [BDMA] based NSAIDs ILs through rat skin in 5 hrs. The cumulative drug permeation trend of [BTEA] based NSAIDs ILs was observed in the order of: [BTEA] [Ibu] (2.02%± 0.96) > [BTEA] [Diclo] (0.165%± 0.005). It was observed that [BTEA] [Ibu] (2.02%± 0.96) showed 12 fold enhancement in cumulative drug permeation in 5 hrs compared to [BTEA] [Diclo] (0.165%± 0.005). The amount of drug permeated in case of [BDMA] based NSAIDs ILs was found in the following order [BDMA] [Diclo] (0.037%±0.007) > [BDMA] [Ibu] (0%). However the flux value of [BDMA] based NSAIDs ILs was considerably lower than that of [BTEA] based NSAIDs ILs. This may be partly attributed to

the difference in thermodynamic activity of anionic APIs due to difference in lipophilicity, solubility of anionic drugs and to higher viscosity and higher molecular weight. The impact of anions on skin permeation could be understood by comparing the octanol-water partition coefficient and molecular weights of benzalkonium API-ILs. As can be seen in Figure.4.17, the trend observed for [BTEA] based NSAIDs ILs indicates that the increase in molecular weight of anion/IL matrix results in decrease in skin permeation. This could be correlated to the reduced interactions between the counterions due to increasing delocalisation of charge and size of the counterion (Freire et al. 2007; Alves et al. 2013). On the other hand the trend observed in case of [BDMA] based NSAIDs ILs was quite different, as can be seen in Figure 4.17.

Comparing the skin permeation of [BTEA] and [BDMA] based NSAIDs ILs, it appears that [BTEA] based drug salts showed enhanced skin permeation profiles compared with [BDMA] based drug salts which could be attributed to the formation of larger ion pairs or clusters with sufficient hydrophobicity and smaller molecular volume.

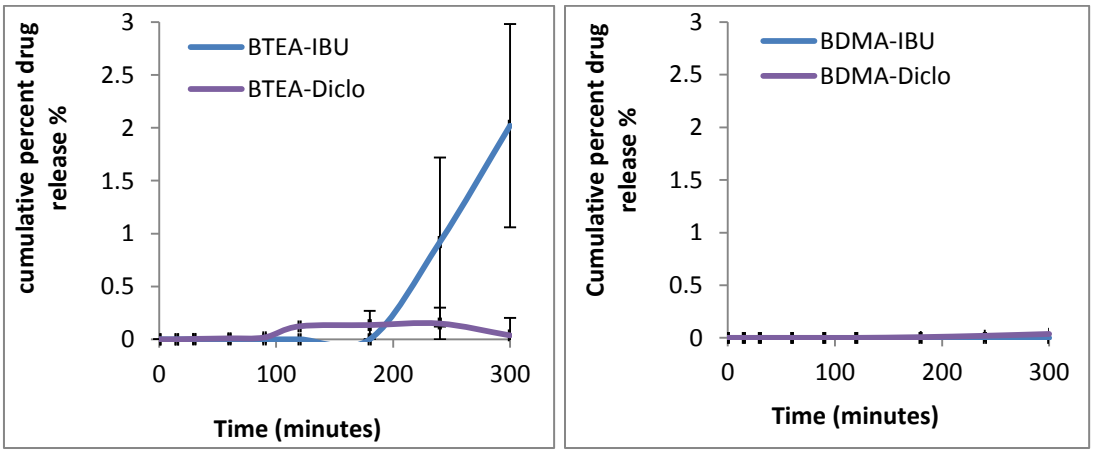


Figure 4.17 Skin permeation profiles of [BTEA] and [BDMA] based NSAIDs ILs (Error bars - standard deviation)

Most of the results are simply based on the partition coefficient and molecular weight measured. It is clear that only [BTEA] [Ibu] could be detected in the receptor compartment based on its low molecular weight than 500 and the lower Ko/w value which is also reflected on the low percentage drug deposition in the different skin layers. On the other side [BTEA] [Diclo] should have similar profile but due to the higher molecular weight the permeation was very limited. As for both [BDMA] [Ibu] and [BDMA] [Diclo], their very high partition coefficient as well as the large molecular weight enhanced to great extent their lipophilicity and partitioning into the different skin layers especially the lipophilic SC layer and minimized their permeation to negligible values which makes them very suitable for topical applications.

This study showed the influence of counterions on skin deposition and permeation after application of neat benzalkonium based NSAIDs ILs on rat skin. The skin deposition and permeation data of studied benzalkonium based NSAIDs ILs suggest that these systems show drug retention which may be due to a number of reasons. First, ILs are entirely composed of cations and anions on equimolar basis. The presence of API in high concentration in IL formulation functions as a driving force to enhance the rate of drug delivery via skin. Second, benzalkonium has a cationic amino group and the chosen drugs have an anionic carboxyl (ibuprofen, diclofenac) group. The interaction between the ionic groups of benzalkonium and drugs helps to reduce or neutralize electrostatic charges and consequently generate a neutralized complex, which might have increased hydrophobicity of drugs. The ILs formed by mixing equimolar quantities (1:1) created neutralized IL complexes which influence the skin permeation of APIs. The low melting point of benzalkonium based API-ILs resulted in increased saturation solubility of drug, which is an important factor

governing thermodynamic activity of drug. Third, the flux values of APIs can be again attributed to the benzalkonium cation moiety in the synthesised API-ILs, which has been reported to have a penetration enhancing effect. Studies have reported the use of quaternary ammonium compounds containing quaternised nitrogen atom as effective skin penetration enhancers (Ahad et al. 2009) (Goindi et al. 2014). In addition to that, quaternary ammonium compounds have been used as penetration enhancers for transbuccal and transnasal drug delivery systems, such as nasal vaccinations. The ability of these compounds to disrupt the lipid bilayer of stratum corneum and penetrate into the skin has been widely used in drug delivery systems such as liposomes (Klinguer et al. 2001; Hough-Troutman et al. 2009). Fourth, the skin permeability of any molecule largely depends on its octanol-water partition coefficient and molecular weight. Benzalkonium drug salts exhibited octanol-water partition coefficient ranging from 0.78(±0.27) to 6.09 (±0.24) indicating that the value of octanol-water partition coefficient was influenced by the counterions. Skin deposition data of studied benzalkonium based NSAIDs ILs suggests that these systems display drug retention and follows direct relationship between drug deposition and molecular weight. On the other hand, permeation data delineated an inverse relationship between flux values and molecular weight of the permeant. Some other molecular properties for example hydrogen bonding to weak Van der Waals forces and drug binding, might be involved and can affect drug delivery via the skin.

However the mechanism behind the skin permeation of APIs through ILs is not yet clear, because ILs are formed by equimolar ratios therefore it is believed that both counterions remain together in the skin as clusters or ion pairs instead of individual solvated ions. This could be justified by the observed electrical

conductivity data. This is also supported by other studies which illustrates that ILs do not dissolve to form independent ions but they form a nanostructured organization in aqueous solution (Nama et al. 2006; Jiang et al. 2007) and by spectroscopic investigations such as NMR techniques (Canongia Lopes et al. 2006; Canongia Lopes and Padua 2006). The focus of this study has been on the application of benzalkonium based NSAIDs ILs to promote the topical drug delivery of ibuprofen and diclofenac.

4.3 Conclusions

The results of this study suggest that prepared benzalkonium based NSAIDs ILs was found to enhance the lipophilicity/hydrophilicity of ibuprofen, diclofenac. Benzalkoium cation acted as penetration enhancer and surfactant, forming ILs combined with ibuprofen and diclofenac in equimolar ratio (1:1). Skin deposition data of studied benzalkonium based NSAIDs ILs suggests that these systems show drug retention following direct relationship between drug deposition and molecular weight. On the other hand, permeation data followed an inverse relationship between flux values and molecular weight of the permeant. The data reported here supports the potential application of IL in topical drug delivery.

Chapter 5

Benzalkonium-sulfacetamide Ionic liquids (ILs)

5.1 Introduction

Recently, third generation of ILs has been emerged which can be formed simply by combining active pharmaceutical ingredients (API) with ILs. This third generation ILs provides enhanced properties such as solubility, drug delivery and permeability as compared with corresponding solid pharmaceutical forms (Ferraz et al. 2011). The very well-known problem of polymorphism associated with crystalline API can be avoided by using active drug in the liquid form which dramatically influence drug's solubility and dosage form (Stoimenovski et al. 2010b). However toxicity associated with counter ions of ILs delayed their entry into biosciences (Cruz-Cabeza 2012). Current communications and reviews talk about the toxicity and antimicrobial, antibacterial activity of ILs and their drug delivery performance. Scientists have reported the synthesis and biological activity of a series of 1-alkyl-3-methylimidazolium chloride and 1-alkylquinolinium ionic liquids which have been tested against a panel of clinically significant microbial pathogens, including MRSA and found to be potent, broad spectrum activity which shows that activity depends on the presence of alkyl chain length. According to the authors the long alkyl chain containing ILs can broke the microbial biofilm which protect the microorganism from antibiotics, disinfectants and antiseptics (Carson et al. 2009; Busetti et al. 2010). Recently, Cole and co-workers reported the synthesis of ILs with long alkyl chain quaternary ammonium and ampicillin anion by using metathesis reaction. The prepared ampicillin based ILs were found to possess higher antibacterial activity in most of the cases compared to starting materials (Cole et al. 2011a). Later on antibacterial and antitumor studies have been carried out

with the ILs based on ampicillin as anion while phosphonium, pyrollidinium, ammonium, cholinium and few short alkyl chain imidazolium as cation (Ferraz et al. 2014a; Ferraz et al. 2015).

Sulfacetamide is a sulfonamide antibiotic drug which is prescribed in the form of sodium salt and finds its applications in ophthalmic (Sridhar et al. 2001) and skin (Margolis et al. 2005). Sulfonamide drugs destroy bacteria by blocking the synthesis of folic acid in bacteria (Maren 1976). The complete potential of quaternary ammonium compounds was acknowledged after the synthesis of benzalkonium chloride in 1935, which is found to be possessing wide spectrum against many bacterial strains after characterization of its anti-bacterial properties (Domagk 1935). These newly emerged water soluble quaternary ammonium compounds display anti-bacterial property against not only gram-positive and gram-negative bacteria, but also against protozoa and pathogen species of fungi (Kull et al. 1961). Interestingly these compounds can also act as penetration enhancers for transbuccal and transnasal drug delivery systems such as nasal vaccinations (Klinguer et al. 2001). Later these water soluble quaternary ammonium compounds found potential applications in the fields of disinfectants (Lopes 1986), surfactants (Hayakawa and Kwak 1991), phase transfer catalysis (Makosza 2000), anti-corrosive agents (Hough-Troutman et al. 2009). Certainly, surfactant molecule is responsible to modify the texture of formulation and also helps to stabilize it. In addition to this, surfactants can alter the transport across biological barriers by directly effecting biological membrane (Khossravi 1997; Savić et al. 2010). The antibacterial mechanism of action of quaternary ammonium compounds is based on the disruption of membrane charge distribution and altering cell membrane permeability, which leads to leakage of all components from cytoplasm which is followed by bacterial death

(Arias-Moliz et al. 2015). Thus following the concept of IL to find out the novel antimicrobial molecule with new modes of action, the combination of antibacterial benzalkonium cations with antibiotic sulphonamide was investigated to understand if there is a synergistic biological effect and the effect of cationic counterion benzalkonium on the penetration behaviour of benzalkonium-sulfacetamide for topical treatment.

5.2 Result and discussion

5.2.1 Characterisation of benzalkonium sulfacetamide IL

Benzyltiethylammonium sulfacetamide [BTEA] [Sulfa] and benzyldimethylhexadecylammonium sulfacetamide [BDMA] [Sulfa] are synthesized via metathesis reaction from the corresponding sodium salts of sulfacetamide and halide salts of Benzyltiethylammonium and benzyldimethylhexadecylammonium. Both of the benzalkonium sulfacetamide IL obtained was yellow viscous liquids at room temperature.

The benzalkonium sulfacetamide IL were characterized by ^{1}H NMR, ^{13} C NMR and IR spectroscopy (Figure 5.1 – 5.4). The proton signals for [BTEA] were recorded at 1.37 - 7.74 ppm and included a signal for 9 protons of its terminal methyl groups at 1.37 ppm (t), 6 protons of adjacent methylene groups were found at 3.27 ppm (q), 2 protons for the methylene next to phenyl group observed at 4.53 ppm (s) while the aromatic protons were found at 7.37-7.50 (m). The signals obtained for [BTEA] were compared with the signals of respective sodium salt sulfacetamide confirms the formation of ionic liquids, indicating good stability of active pharmaceutical ingredient during salt formation. This data was further supported by the IR spectroscopy (IR spectra are provided as supplementary Information). Following the metathesis reaction the symmetric and antisymmetric stretching of carboxylic anion was found 1592 $cm^{-1}$and 1295 cm^{-1} (for sulfacetamide).

NMR and FTIR characterization data

Benzyltriethylammonium-Sulfacetamide [BTEA] [Sulfa]: Yield 82%.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.37 (t, 9 H), 1.86 (s, 3 H), 3.27 (q, 6 H), 4.53 (s, 2 H), 6.51 - 6.57 (d, 2 H), 7.37 - 7.50 (m, 5 H), 7.66 – 7.74 (d, 2 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 8.20, 18.44, 27.04, 52.76, 58.23, 76.74, 77.06, 77.26, 77.38, 113.43, 127.19, 128.71, 129.43, 130.68, 132.45

IR (ν_{max} cm^{-1}): 3379, 3212, 2982, 2346, 1648, 1592, 1360, 1295, 1128, 1082, 827.4, 755.6, 683.8, 619.6

Benzyldimethylhexadecylammonium-Sulfacetamide [BDMA] [Sulfa]: Yield 78 %.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.81 - 0.93 (t, 3 H), 1.18 - 1.30 (m, 26 H), 1.85 (s, 3 H), 2.98 (s, 6 H), 4.20 - 4.32 (m, 2 H), 4.57 (s, 2 H), 6.45 - 6.57 (d, 2 H), 7.32 - 7.50 (m, 5 H), 7.70 (d, 2 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 14.12, 22.69, 27.07, 29.29, 29.37, 29.44, 29.51, 29.63, 29.68, 29.71, 31.93, 49.59, 76.72, 77.04, 77.35, 113.70, 128.68, 129.14, 133.23

IR (ν_{max} cm^{-1}): 3339, 2955, 2922, 2853, 1630, 1596, 1362, 1305, 1125, 1084, 828.1, 681.1

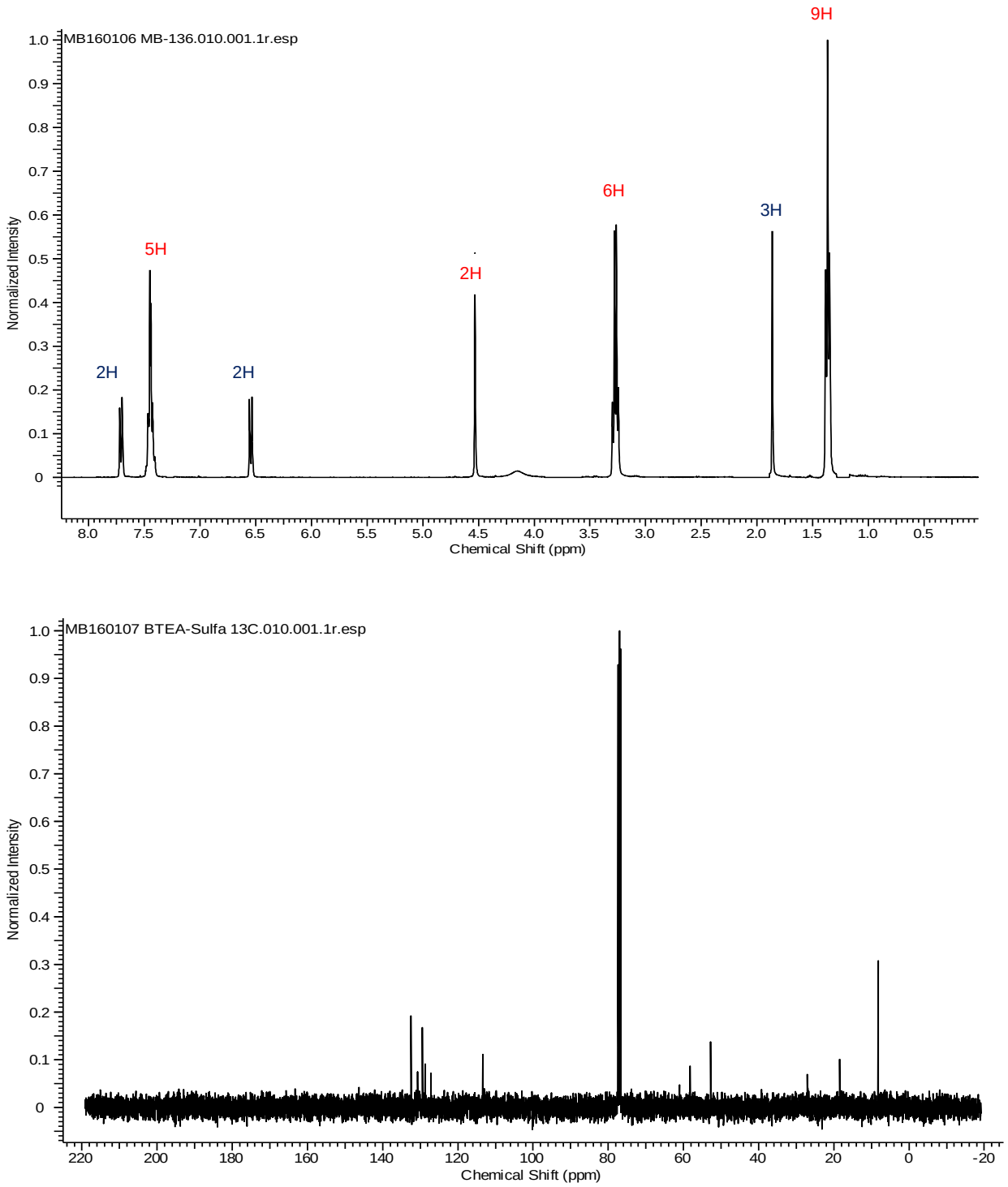


Figure 5.1 ^{1}H and ^{13}C NMR spectra of Benzyltriethylammonium-Sufacetamide [BTEA] [sulfa]

[BTEA] and [Sulfa] protons are represented in red and blue colours respectively

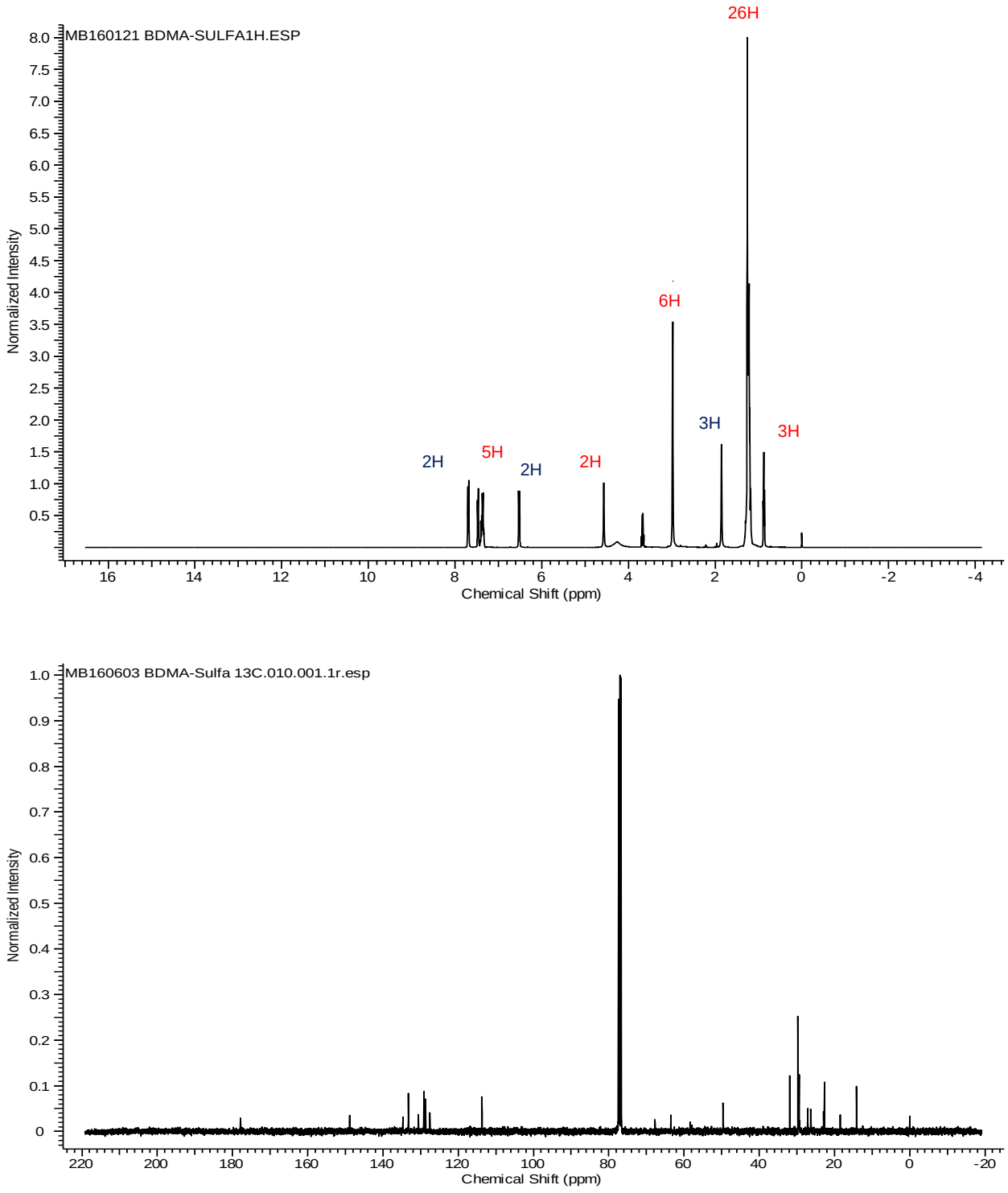


Figure 5.2 ^{1}H and ^{13}C NMR spectra of Benzyltriethylammonium-Sufacetamide [BDMA] [sulfa]

[BDMA] and [Sulfa] protons are represented in red and blue colours respectively

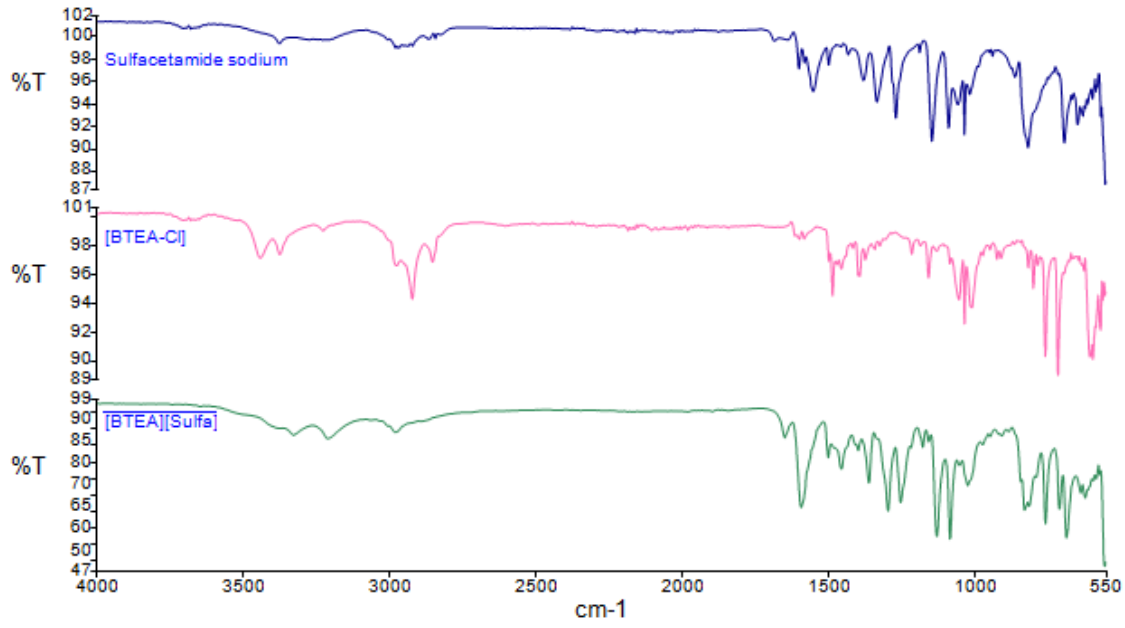


Figure 5.3 FTIR spectra of Benzyltriethylammonium-Sufacetamide [BTEA] [sulfa]

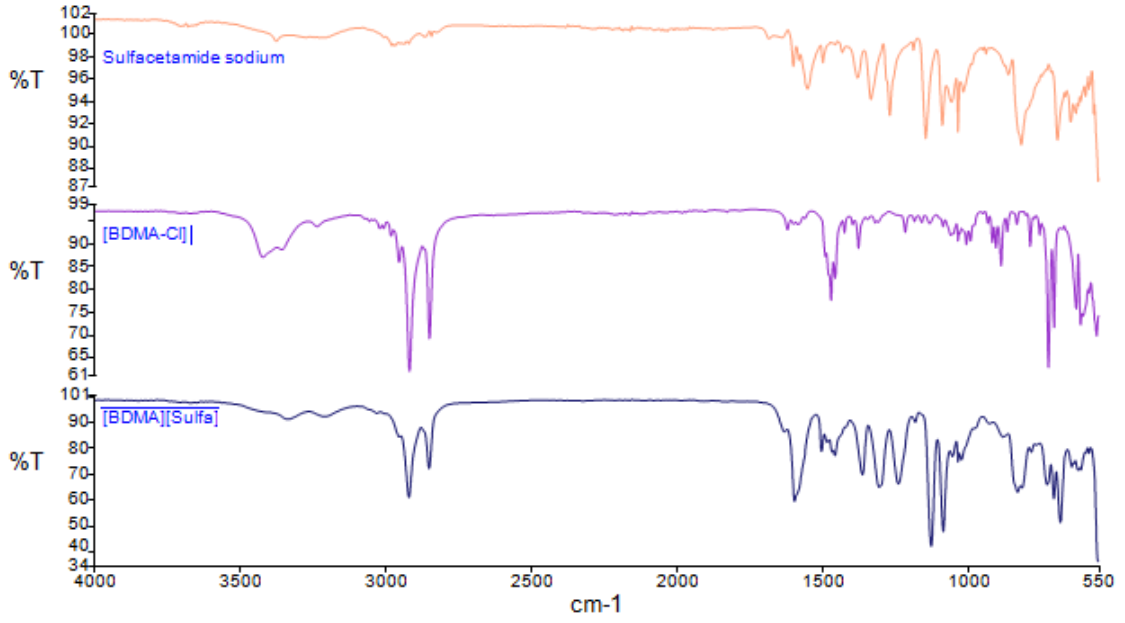


Figure 5.4 FTIR spectra of Benzyldimethylhexadecylammonium-Sulfacetamide [BDMA][Sulfa]

5.2.2 Thermal Behaviour

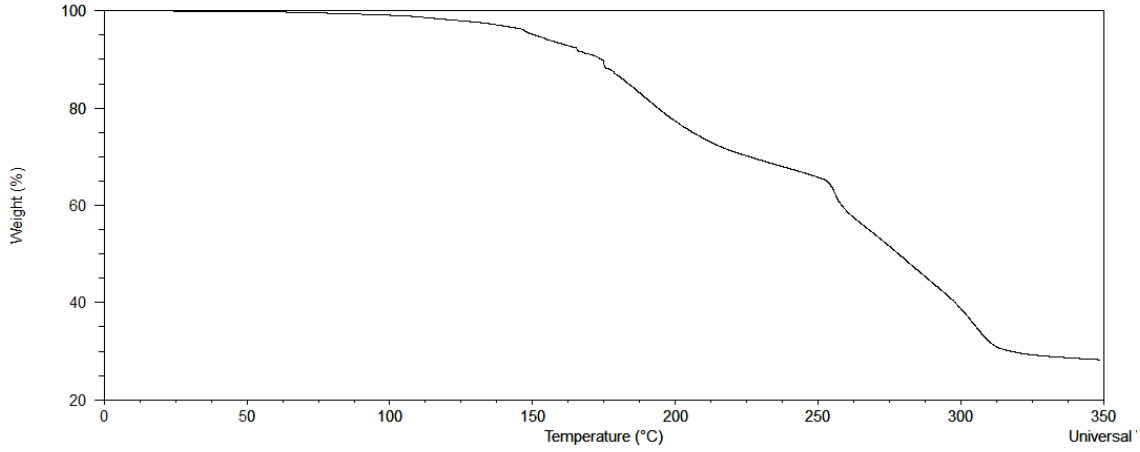
The effect of cation on the thermal properties of [BTEA] [Sulfa] [BDMA] [Sulfa] was studied using DSC and TGA. The synthesized ionic liquids are viscous liquids at room temperature. Table.5.1 shows that the thermal stability of [BTEA] [Sulfa] and [BDMA] [Sulfa] was found to 161.38, 164.94 respectively. Two step decomposition was observed for both [BTEA] [Sulfa] and [BDMA] [Sulfa]. For the TGA and DSC profiles of [BTEA] [Sulfa] and [BDMA] [Sulfa] please refer (Figure 5.5 and 5.6).The impact of cation can be observed by difference in thermal stability of drug salts. When you compare the decomposition temerature of [BTEA] [Sulfa] and [BDMA] [Sulfa] then it was observed that [BDMA] [Sulfa] display higher decomposition temperature. The increase in decomposition temperature could be attributed due to increase in interparticle forces (Van der Waal forces) which arises with increase in alkyl chain length (Canongia Lopes and Padua 2006; Chancelier et al. 2016).

However, the DSC thermograms of [BTEA] [Sulfa] and [BDMA] [Sulfa] systems display no phase transitions in the temperature range studied, which shows that these systems remains amorphous.

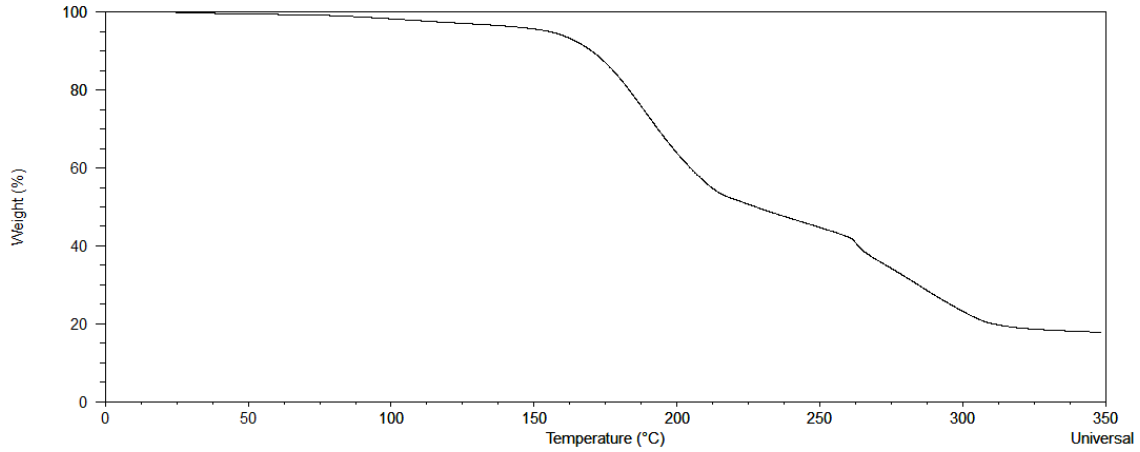
Table 5.1 Physicochemical properties of synthesized benzalkonium sulfacetamide ILs

API-ILs	MW	Ko/w	Td(°C)	% Deposited in stratum corneum	% Deposited in Epidermis	%Deposited in Dermis
[BTEA] [Sulfa]	405.5	0.75(±0.18)	161.38	12.37(±4.2)	5.76(±2.84)	11.09(±9.13)
[BDMA] [Sulfa]	573.8	5.62(±0.57)	164.94	1.42(±0.35)	0.29(±0.14)	0.64(±0.4)

TGA and DSC profiles benzalkonium-sulfacetamide ILs

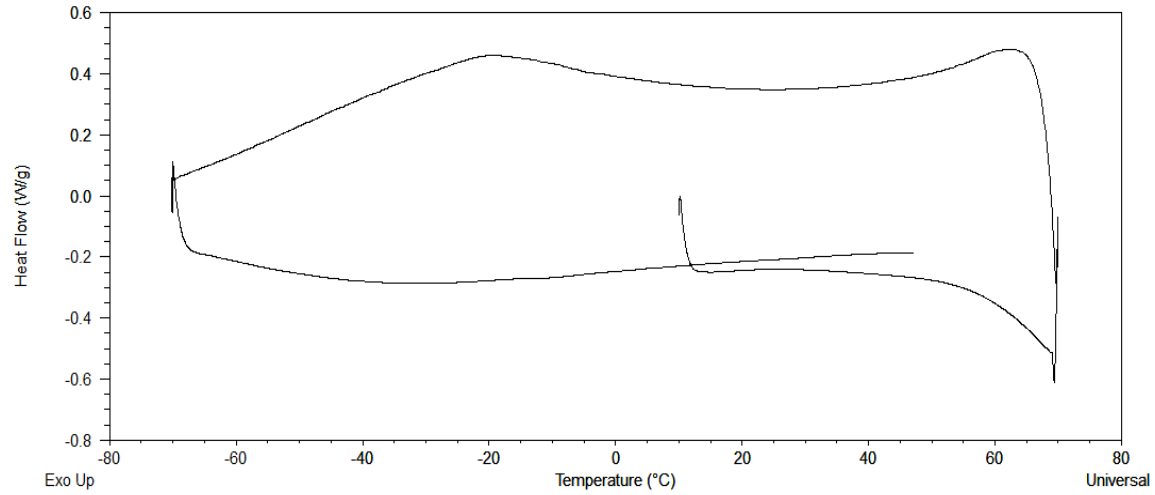


(a)

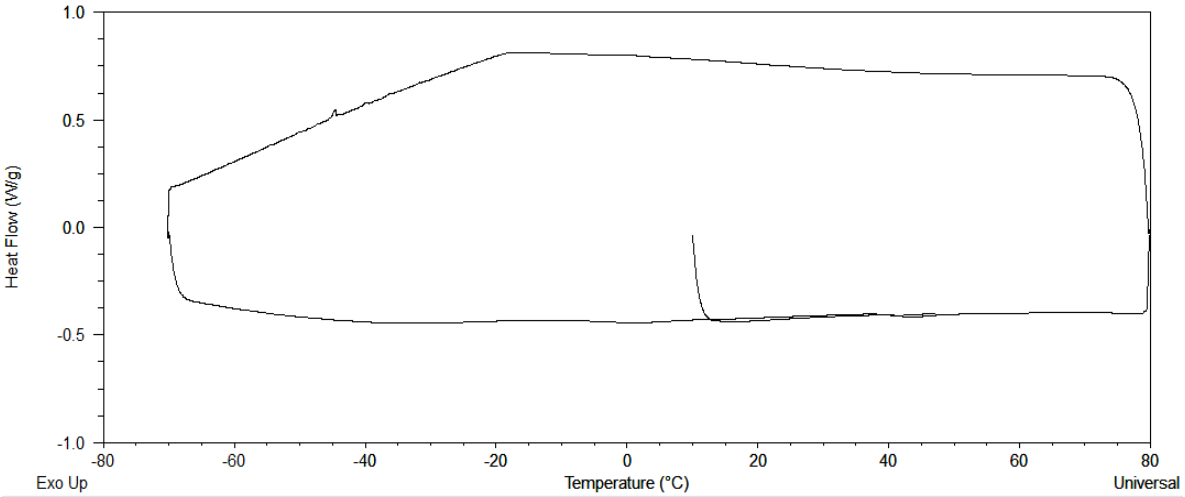


(b)

Figure 5.5 TGA profile of (a) [BTEA][Sulfa] (b) [BDMA][Sulfa]



(a)



(b)

Figure 5.6 DSC profile of (a) [BTEA][Sulfa] (b) [BDMA][Sulfa]

5.2.3 Electrical conductivity studies

The electrical conductivity profiles of aqueous solutions of [BTEA] [Sulfa] and [BDMA] [Sulfa] are studied at high concentration range. The conductivity of [BTEA] [Sulfa] and [BDMA] [Sulfa] is shown in Figure 5.7. The room temperature conductivity values [BDMA] [Sulfa] was found to be lower than [BTEA] [Sulfa], for detailed information please refer back to section 4.2.3.

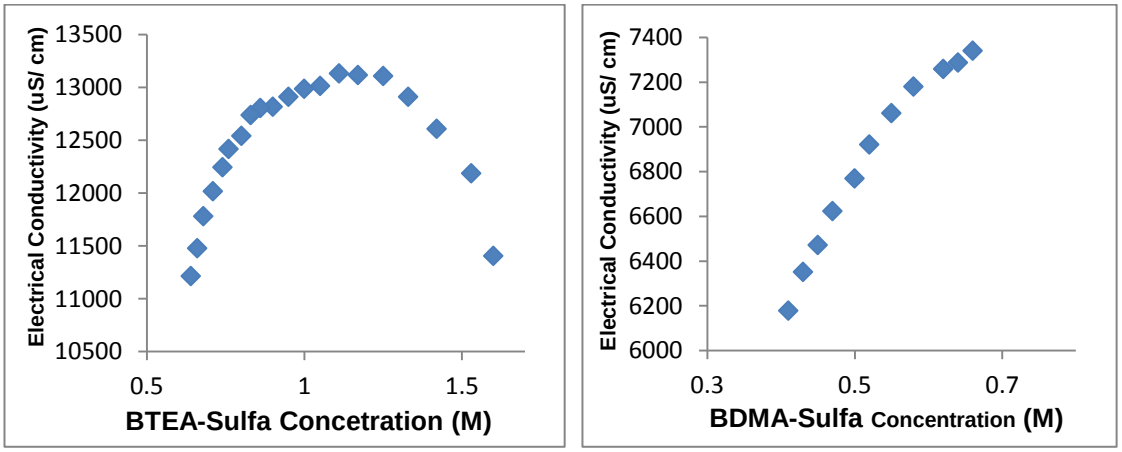


Figure 5.7 Electrical conductivity of aqueous solutions of [BTEA][Sulfa] and [BDMA][Sulfa]

5.2.4 Octanol-water partition coefficient

The octanol-water partition coefficient of [BTEA][Sulfa] and [BDMA][Sulfa] was investigated to understand the passive diffusion across biological membranes. The results as shown in Table.5.1, indicates that the [BDMA][Sulfa] is more lipophilic than [BTEA][Sulfa], which could be attributed due to increase in molecular weight and alkyl chain length on the cation as shown in Figure.5.8. (Domańska et al. 2003; Wu et al. 2003; Crosthwaite et al. 2004).

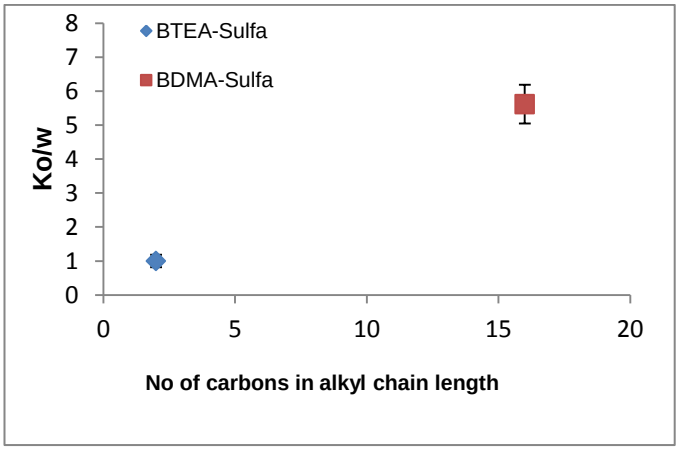


Figure 5.8 Octanol-water partition coefficient of [BTEA][Sulfa] and [BDMA][Sulfa] (Error bars - standard deviation)

5.2.5 Ex-vivo skin studies

Drug deposition study and permeation studies

Ex-vivo permeation and deposition studies were performed to determine the rate and the extent of benzalkonium API-ILs permeated and deposited in the various layers of skin.In order to understand the effect of cation on the skin deposition and permeation values, the amount of drug content in stratum corneum, epidermis and dermis was evaluated after the ex-vivo skin permeation experiment through rat skin. The skin deposition and permeation values can be seen in Table.5.1 and Figure.5.9.

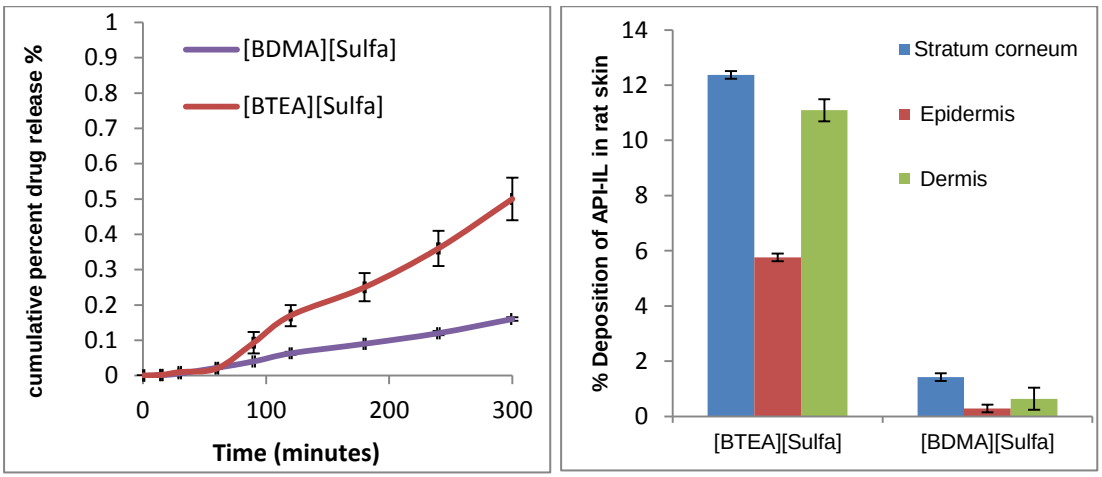


Figure 5.9 skin deposition profiles and permeation profiles of [BTEA][Sulfa], [BDMA][Sulfa] (Error bars - standard deviation)

It was observed that permeation and skin deposition values of [BDMA] [Sulfa], was found to be lower than [BTEA] [Sulfa] in all the layers of skin. The results are based on molecular weight and partition coefficient of the synthesised benzalkonium sulfacetaminde ILs. As can be seen from Table.5.1 the molecular weight and partition coefficient of [BDMA][Sulfa] is higher compared to [BTEA][Sulfa]. The increase in molecular weight influences the diffusion coefficient of the [BDMA][Sulfa] with increasing bulky molecule leading to decrease in diffusivities (Crank 1975). Scheuplein et al. suggested that small molecule with lower molecular weight cross human skin more faster rate compared to large molecule (Scheuplein et al. 1969). The skin deposition data of studied benzalkonium sulfacetamide ILs suggest that these systems show drug retention and decrease in skin permeability with increase in molecular weight. As it is clear from results that [BDMA][Sulfa] penetrates much slower than [BTEA][Sulfa]. The formulation with desired properties can be generated by combining both the studied ILs to tailor the penetration and deposition properties of sulfacetamide for topical applications.

5.2.6 Antibacterial efficacy studies

5.2.6.1 Agar diffusion results

Antibacterial activity of the starting materials and product ILs was determined by measuring the diameters of the zones of inhibition surrounding the agar diffusion discs saturated with 20 µg, 30 µg, 40 µg, 50 µg and 100 µg/ml of the test compounds. Disc diffusion results of the studied ILs and parent compounds could be referred in (Figure 5.10).

The formation of zones of inhibition shows the interaction between the bacteria trying to grow on the agar surface and the diffusing test molecule. No antibacterial activity is confirmed when the bacterial growth occurs upto the edge of the disc. The results of the diffusion experiment are shown in Table 5.2. The lack of antibacterial activity was represented by a diameter of “0 mm” in the table.

Table 5.2 Disc diffusion results of benzalkonium sulfacetamide IL and parent components

Bacterial strain	Gram	[BDMA-Cl] (µg/ml)					[BTEA-Cl] (µg/ml)					[Sod-Sulfa] (µg/ml)				
		20	30	40	50	100	20	30	40	50	100	20	30	40	50	100
Staphylococcus aureus	+	7	10	10.5	11.6	12	0	0	0	0	0	0	0	0	0	0
Escherichia coli	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Bacterial strain	Gram	[BDMA][Sulfa] (µg/ml)					[BTEA][Sulfa] (µg/ml)				
		20	30	40	50	100	20	30	40	50	100
Staphylococcus aureus	+	7	10.5	11	12	14	0	0	0	0	0
Escherichia coli	-	0	0	0	0	0	0	0	0	0	0

Zone diameters are reported in mm

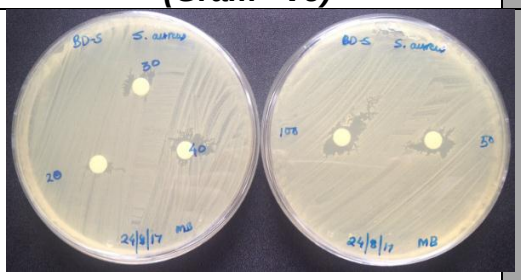
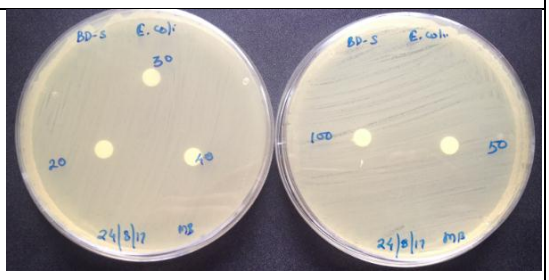
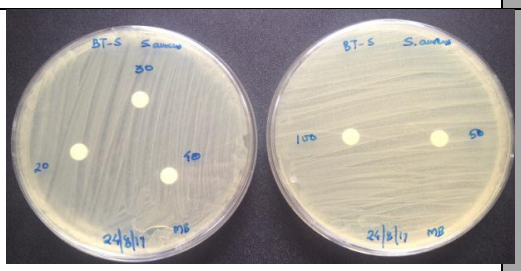
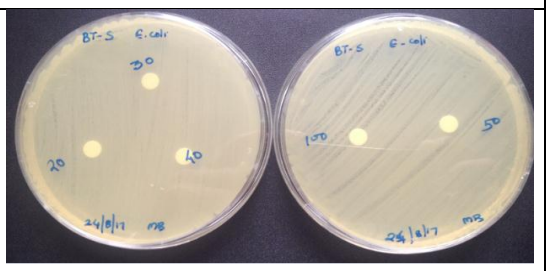
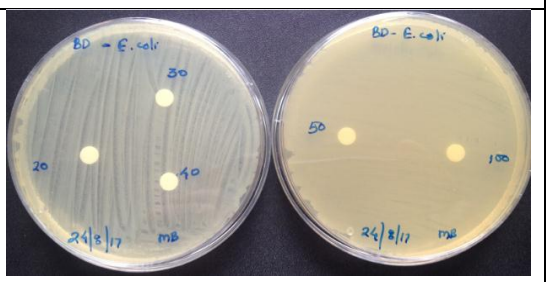
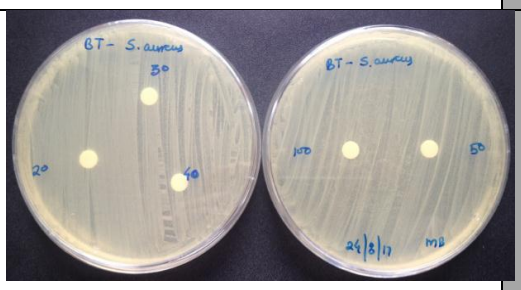
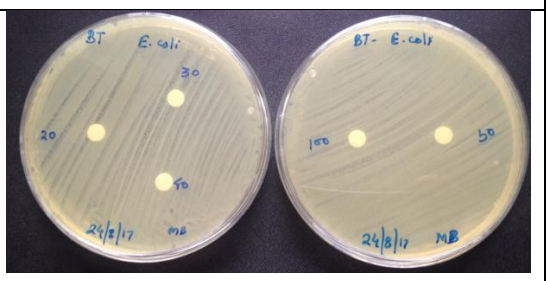
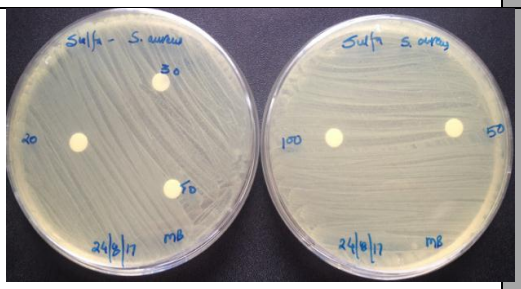
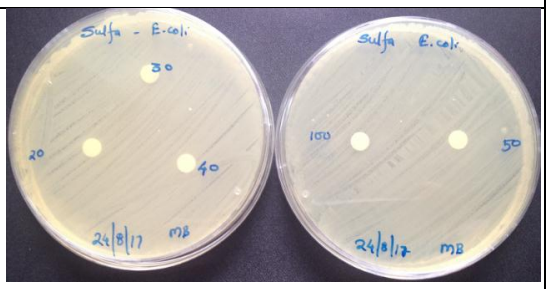
	Staphylococcus aureus (Gram +ve)	*Escherichia coli (Gram –ve)*
[BDMA] [Sulfa]		
[BTEA] [Sulfa]		
[BDMA-Cl]		
[BTEA-Cl]		
[Sod-Sulfa]		

Figure 5.10 Antibacterial activity of benzalkonium sulfacetamide ILs and parent components (agar diffusion results)

The screening results of agar diffusion test suggest that only two compounds [BDMA-Cl] and [BDMA-Sulfa] was found to display antibacterial efficacy against *staphylococcus aureus* at studied concentration. However here was no activity with [BTEA-Cl], [Sod-sulfa] and [BTEA-Sulfa]. On the other hand the lack of antibacterial efficacy was found against *E.coli* by all the five tested compounds. These parent compounds along with synthesised benzalkonium-sulfacetamide ILs were further investigated in minimum inhibitory concentration (MIC) assays to quantify their antibacterial efficacy against the studied bacterial strains.

5.2.6.2 MIC determination studies

The antibacterial activity of [BDMA][Sulfa] and [BTEA][Sulfa] IL along with the starting material was studied by minimum inhibitory concentration (MIC) against *staphylococcus aureus* (Gram positive bacteria) and *E.coli* (Gram negative bacteria) to investigate if there is synergistic effect. MIC can be defined as the lowest concentration of antibiotic which shows no visible growth. Growth of these bacteria in terms of optical density was measured by spectrophotometer at 570 nm after 24 hours. Control measurements were performed without the addition of test sample and the optical density was found to be 0.497(±0.040) for *staphylococcus aureus* (Table 5.3). The optical density of the test compounds was found to be decreased as the concentration was decreased. The turbidity was observed at higher concentration in case of [BDMA][Sulfa] and [BDMA-Cl] which is due to the sample itself as no bacterial growth was observed during plating.

Percentage growth inhibition data of product IL and the starting materials suggests that inhibition increases with increase in concentration of the test

compound. Table 5.3shows the percentage growth inhibition of test results against *Staphylococcus aureus* (Gram-positive bacteria) which indicates that 100 % growth inhibition was obtained by [BDMA-Cl] and [Sod-Sulfa] at 0.97 µg/ml and 125 µg/ml respectively when used alone. The combination of both in the form of [BDMA][Sulfa] IL showed 100 % growth inhibition at 1.95 µg/ml concentration. On the other hand, [BTEA-Cl] and [Sod-Sulfa] were found to inhibit bacterial growth at 2500 µg/ml and 125 µg/ml respectively, when tested alone. In case of [BTEA][Sulfa] IL as combination 100 % growth inhibition was observed at 156.25 µg/ml. Based on the plating, the [BDMA][Sulfa] and [BDMA-Cl] percentage growth inhibition formula cannot be followed due to turbidity of the test samples themselves.

Thus comparing the antibacterial efficacy of synthesised [BDMA][Sulfa] and [BTEA][Sulfa] ILs with starting compounds, sodium sulfacetamide, benzalkonium halides it was found that the antibacterial activity of products was not higher than that of the parent materials. However, comparing the antibacterial activity of [BDMA][Sulfa] and [BTEA][Sulfa] IL, the [BDMA][Sulfa] was found to be more effective which could be correlated to the presence of long alkyl chain length associated to cation (Smiglak et al. 2014).

Similar trend was observed regarding the antimicrobial efficacy of the compouds against *E.coli* (Gram-negative bacteria). The optical density of the control, test samples and percentage inhibition growth is summarised in Table 5.4.

However, [BDMA][Sulfa] and [BTEA][Sulfa] IL were found to be more effective against *staphylococcus aureus* (Gram positive bacteria) as compared to *E.coli* (Gram negative bacteria). This could be attributed to the difference in the structure of cell wall of Gram positive and Gram negative bacteria. A very thick

layered cell wall composed of peptidoglycan is found in Gram positive bacteria while Gram negative bacteria possess very thin cell wall. Gram negative bacteria possess an outer membrane which acts as permeability barrier whereas Gram positive bacteria lack this outer membrane. The MIC and MBC values are shown in Table 5.5 and Table 5.6 respectively. The plating for MBC results of studied compounds against *staphylococcus aureus* and *E.coli* are presented in (Figure 5.11).

Thus after observing the data of MIC, MBC, optical density and percentage growth inhibition of the synthesised ILs and the starting materials it can be said that no synergistic effect was observed in case of ILs. This can be explained as follows. The antibacterial mechanism of action of sulfonamide drug molecule is based on the blocking of the synthesis of folic acid in bacteria leading to bacterial death (Maren 1976). While quaternary ammonium compounds function via disruption of membrane charge distribution leading to alter the cell membrane permeability. This results to drowning out cytoplasmic components and finally bacterial death (Arias-Moliz et al. 2015). In combination, it is likely because of the different antibacterial mechanism action of both antibacterial agents may compete to access the site of activity within the bacterial cell.

Table 5.3 Optical density and % growth inhibition of benzalkonium-sulfacetamide ILs determined at 570 nm

***Staphylococcus aureus* (Gram-positive bacteria)**											
Concentration (µg/ml)	**[BDMA][Sulfa]**		**[BDMA-Cl]**		**[Sod-Sulfa]**		Concentration (µg/ml)	**[BTEA][Sulfa]**		**[BTEA-Cl]**	
	*OD	%*GI	OD	% GI	OD	% GI		OD	% GI	OD	% GI
Control	0.497(±0.04)	-		-		-			-	-	-
1000	0.409	100	0.253	100	-0.024	102.4	2500	-0.026	102.6	0.105	89.5
500	0.182	100	0.349	100	-0.02	102	1250	-0.022	102.2	0.251	74.9
250	0.165	100	0.253	100	-0.018	101.8	625	-0.02	102	0.345	65.5
125	0.06	100	0.133	100	-0.014	101.4	312.5	-0.019	101.9	0.462	53.8
62.5	-0.005	100.5	0.036	100	0.051	94.9	156.25	-0.007	100.7	0.517	48.3
31.25	-0.02	102	-0.01	101	0.323	67.7	78.125	0.295	70.5	0.488	51.2
15.62	-0.024	102.4	-0.017	101.7	0.481	51.9	39.0625	0.433	56.7	0.562	43.8
7.81	-0.018	101.8	-0.022	102.2	0.462	53.8	19.5312	0.481	51.9	0.55	45
3.9	-0.018	101.8	-0.023	102.3	0.461	53.9	9.76562	0.468	53.2	0.475	52.5
1.95	-0.011	101.1	-0.021	102.1	0.491	50.9	4.88281	0.455	54.5	0.469	53.1
0.97	0.092	90.8	-0.022	102.2	0.465	53.5	2.44140	0.459	54.1	0.365	63.5
0.48	0.099	90.1	0.056	94.4	0.475	52.5	1.22070	0.462	53.8	0.359	64.1
0.24	0.275	72.5	0.116	88.4	0.478	52.2	0.61035	0.462	53.8	0.269	73.1
0.12	0.659	34.1	0.367	63.3	0.845	15.5	0.305	0.861	13.9	0.894	10.6

OD: Optical density *GI: Growth inhibition

Table 5.4 Optical density and % growth inhibition of benzalkonium-sulfacetamide ILs determined at 570 nm

***E.coli* (Gram-negative bacteria)**											
Concentration (µg/ml)	**[BDMA][Sulfa]**		**[BDMA-Cl]**		**[Sod-Sulfa]**		Concentration (µg/ml)	**[BTEA][Sulfa]**		**[BTEA-Cl]**	
	*OD	%*GI	OD	% GI	OD	% GI		OD	% GI	OD	% GI
Control	0.674 (±0473)	-		-		-			-		
1000	-0.034	103.4	0.252	100	-0.028	102.8	2500	0.055	94.5	0.466	53.4
500	0.32	100	0.326	100	-0.027	102.7	1250	-0.022	102.2	0.65	35
250	0.127	100	0.24	100	-0.023	102.3	625	-0.021	102.1	0.681	31.9
125	0.074	100	0.138	100	-0.021	102.1	312.5	0.049	95.1	0.719	28.1
62.5	0	100	0.036	100	0.354	64.6	156.25	0.144	85.6	0.81	19
31.25	-0.02	102	-0.011	101.1	0.503	49.7	78.125	0.371	62.9	0.717	28.3
15.62	-0.025	102.5	-0.016	101.6	0.385	61.5	39.0625	0.318	68.2	0.734	26.6
7.81	-0.021	102.1	-0.024	102.4	0.433	56.7	19.5312	0.498	50.2	0.796	20.4
3.9	0.635	36.5	-0.024	102.4	0.685	31.5	9.76562	0.665	33.5	0.657	34.3
1.95	0.732	26.8	0.648	35.2	0.787	21.3	4.88281	0.721	27.9	0.678	32.2
0.97	0.804	19.6	0.696	30.4	0.88	12	2.44140	0.661	33.9	0.697	30.3
0.48	0.646	35.4	0.67	33	0.71	29	1.22070	0.703	29.7	0.679	32.1
0.24	0.638	36.2	0.651	34.9	0.647	35.3	0.61035	0.722	27.8	0.681	31.9
0.12	0.989	1.1	0.788	21.2	0.978	2.2	0.30517	0.991	0.9	0.941	5.9

OD: Optical density *GI: Growth inhibitions

The minimum inhibitory concentration (MIC) (Table 5.5) and minimum bactericidal concentration (MBC) values (Table 5.6) were determined for [BDMA-Cl], [BTEA-Cl], [Sod-Sulfa], [BDMA][Sulfa] and [BTEA][Sulfa].

Table 5.5 MIC Values[a]

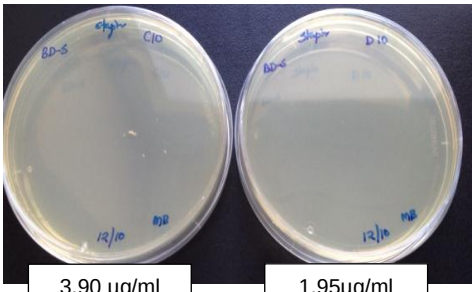
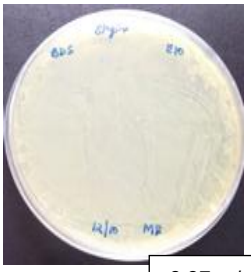
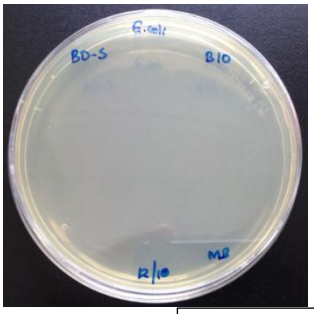
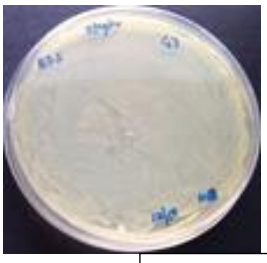
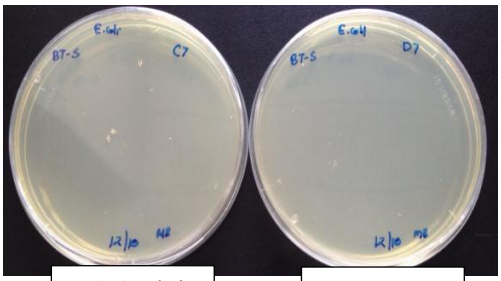
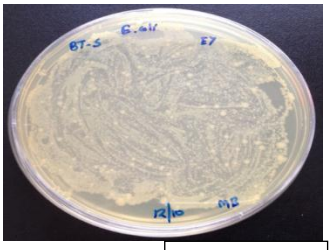
Strain	Starting materials			Ionic liquids	
	[BDMA-Cl]	[BTEA-Cl]	[Sod-Sulfa],	[BDMA][Sulfa]	[BTEA][Sulfa]
Staphylococcus aureus	0.97	>2500	125	1.95	156.25
E.coli	3.9	>2500	125	7.81	625

[a]In µg/ml

Table 5.6 MBC Values[a]

Strain	Starting materials			Ionic liquids	
	[BDMA-Cl]	[BTEA-Cl]	[Sod-Sulfa],	[BDMA][Sulfa]	[BTEA][Sulfa]
Staphylococcus aureus	0.97	>2500	~300	1.95	156.25 - 350
E.coli	7.81	>2500	~300	7.81	625

[a]In µg/ml

	Staphylococcus aureus (Gram +ve)		***Escherichia coli (Gram –ve)***
[BDMA] [Sulfa]	 3.90 µg/ml 1.95µg/ml  0.97µg/ml		 7.81µg/ml
[BTEA] [Sulfa]	 78.12µg/ml		 1250µg/ml 625µg/ml  312.5µg/ml

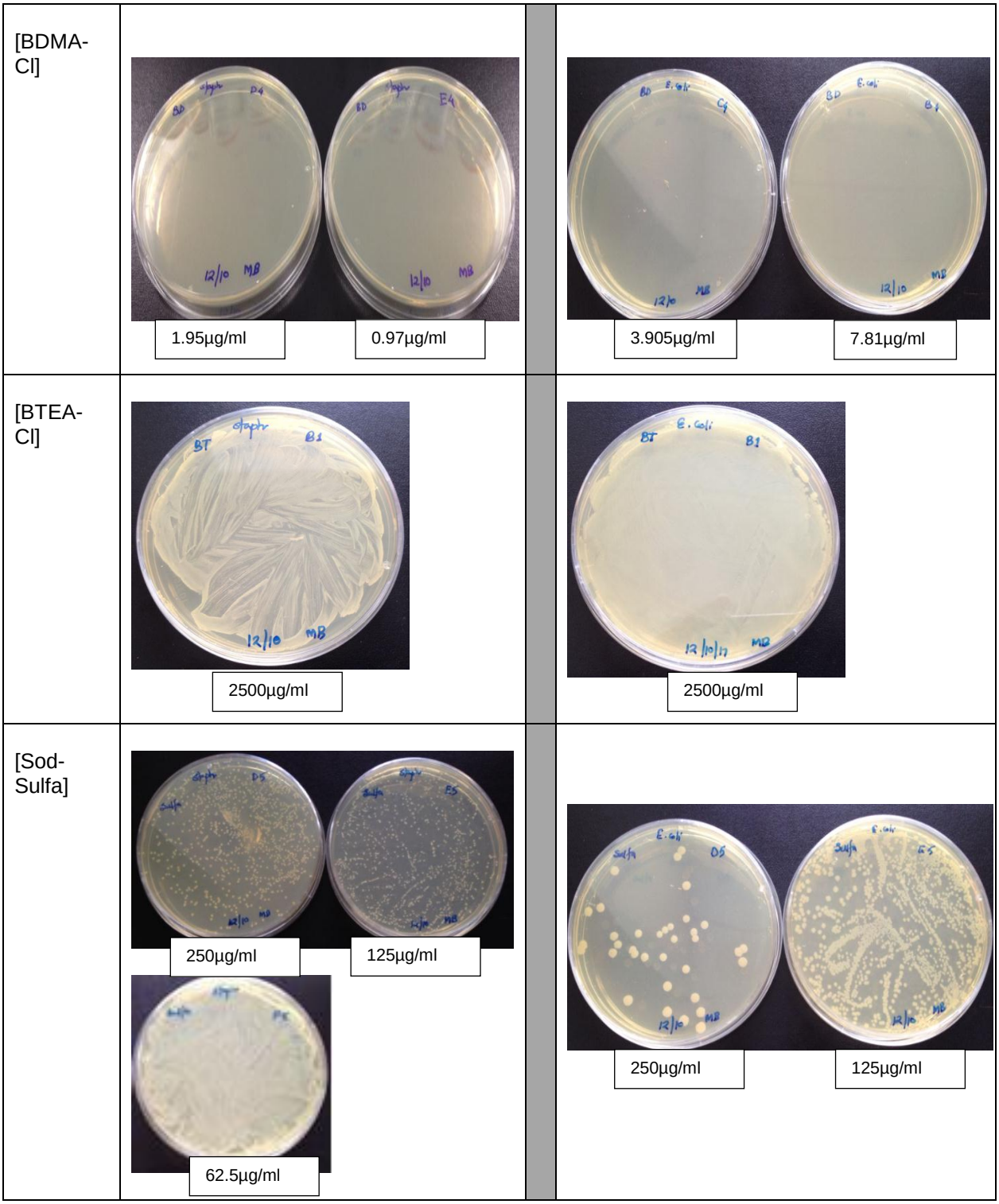


Figure 5.11 Antibacterial activity of benzalkonium sulfacetamide ILs and parent components (Broth dilution method)

5.3 Conclusions

The results of this study suggest that prepared benzalkonium sulfacetamide ILs were found to enhance the lipophilicity/hydrophilicity. Benzalkoium cation acted as penetration enhancer and surfactant, forming ILs combined with sulfacetamide in equimolar ratio (1:1). Skin deposition and permeation data of studied benzalkonium sulfacetamide ILs suggests that these systems show drug retention following an inverse relationship between flux values and molecular weight. On the other hand, antimicrobial activity of product ILs showed no synergism compared with the starting materials.

Chapter 6

Benzalkonium based mixed anion Ionic liquid (ILs)

6.1 Introduction

The objective of this chapter is to understand the permeation behaviour of ibuprofen and salicylic acid in different forms (salt and hydrogen bonded neutral molecule) that exist in benzalkonium based mixed anion ILs, through an artificial membrane. In addition to this the effect of cationic counterion on the permeation profiles of ibuprofen and salicylic acid was also assessed. The data obtained in chapter 4 indicated that it would be of interest to evaluate the effect of hydrogen/ionic bonding on permeation properties of three component system. With increasing understanding about the biocompatibility and toxicity of Ionic liquids, they have been discussed as a formulation concept of active pharmaceutical ingredients (APIs) and enlarged into pharmaceutical applications (Hough et al. 2007a).The degree of ionocity and molar ratio of counterions are crucial characteristics for pharmaceutical companies as these elements are important for governing bioavailability of active pharmaceutical ingredients including solubility, absorption, distribution, metabolism and finally excretion (Kelley et al. 2013). Studies have been reported which demonstrate that not only ionic liquids but deep eutectics, oligomeric ionic liquids and ILs with mixed anions also possess wide range of degree of ionocity (Bica and Rogers 2010; Bica et al. 2011; Kelley et al. 2013).

Macfarlane et al*.* reported the formation of dimeric and oligomeric ions when N-methylpyrolidone reacted with acetic acid and showed that this system found to display highest ionocity due to formation of oligomeric anionic

species $[(AcO)_3H_2]^-$ stabilised by hydrogen bonds (Johansson et al. 2008). Oligomeric anions can also be found in Lewis acidic ILs which are composed of monomeric, dimeric and mixed-valent anions for example $AlCl_4^-$, $Al_3Cl_7^-$ or for halides such as iodides complexed with oligomeric species based on $[I_3^-]$, $[I_5^-]$, and $[I_7^-]$ (Wicelinski et al. 1988; Bica and Rogers 2010). The formation of oligomeric cations or anions which are stabilised by hydrogen bond have been reported by *Rogers* et al*.* by simple mixing of pharmaceutically ionic liquid with excess solid or base (Bica and Rogers 2010). Hui Wang et al reported the simultaneous transport of ibuprofen and lidocaine as a single compound through membrane which are held together by hydrogen bonds or partially ionized interactions (Wang et al. 2014).

Benzalkonium chloride displays anti-bacterial properties against not only gram-positive and gram-negative bacteria, but also against protozoa and pathogen species of fungi (Kull et al. 1961). As discussed in Chapter 1, Ibuprofen is a non-steroidal anti-inflammatory drug which is used to treat pain and inflammation in rheumatic disease and other musculoskeletal problems. Topical administration of ibuprofen not only allows overcoming the drawbacks associated with oral administration but also helps in achieving faster pain relief (Tegeder et al. 1999)**.** Salicylic acid possess broad range of properties such as anti-inflammatory, analgesics, antiseptic, preservative and key ingredients of skin care products (Wu 2007). Stratum corneum is the outermost layer of the skin which forms the barrier for most of the drug molecule to be absorbed via skin as only a limited number of drugs display the optimal physicochemical characteristic to permeate through skin to exert desired therapeutic effect (Wotton et al. 1985).

Ionic liquefied APIs could be formulated in two different ways: (1) combining an ionisable drug with a proper counter ion or (2) by combining two pharmaceutically active ionisable drugs (Hough and Rogers 2007; Stoimenovski et al. 2010a). The formation of hydrogen bonding between cation and anion can be easily nullified by utilising the former approach therefore benzalkonium based NSAIDs ILs are prepared via anion exchange reaction by mixing benzalkonium chloride and sodium salt of drugs (ibuprofen, salicylic acid) in equimolar ratio (1:1). And then benzalkonium based mixed anion ILs were prepared by simple mixing of benzalkonium based NSAIDs ILs with other acidic active pharmaceutical ingredient. One of the key challenges with these benzalkonium based mixed anion ILs moieties is to look into whether these moieties are enough bounded/interacting that they remain intact and affect membrane transport. Therefore it is interesting to look into the permeation profiles of ibuprofen and salicylic acid in different form (salt or hydrogen bonded neutral molecule) exist in IL with mixed anions through artificial membrane. In addition to this the effect of cationic counterion on the permeation profiles of ibuprofen and salicylic acid, thermal, octanol-water partition coefficient and conductivity properties was also evaluated.

6.2 Results and discussion

6.2.1 Characterisation of benzalkonium based mixed anion ILs

The benzalkonium based mixed anion ILs were characterized by ^{1}H NMR, and ^{13}C NMR (Figure 6.1 - 6.5). The proton signals for benzyltriethylammonium cation of M1 were recorded at 1.16 - 7.31 ppm and included a signal for 9 protons of its terminal methyl groups at 1.16-1.28 ppm (t), 6 protons of adjacent methylene groups were found at 2.99-3.11ppm (q), 2 protons for the methylene next to phenyl group observed at 4.26 ppm (s) while the aromatic protons were found at 7.19-7.31 (m). The signals obtained for benzyltriethylammonium cation were compared with the signals of ibuprofenate salicylic acid anion confirming the formation of ionic liquid with mixed anions and indicating good stability of active pharmaceutical ingredient (Table 6.1).

NMR spectroscopy has the potential to demonstrate the presence of hydrogen bonding. The standard temperature range of the Bruker Avance III 400 spectrometer operating at 400 MHz NMR equipment is 278 K – 333 K. These limitations do not allow measurements below 278 K, temperature therefore VT (variation temperature) NMR spectroscopy analysis was performed on M1 (benzyltriethylamonium-ibuprofenate salicylic acid) in deuterated chloroform from 298.15 K to 278.15 K at a decreasing rate of 5 K to see the change in the chemical shift of delocalised proton. The result shows downfield shift of delocalised acidic proton with decrease in temperature. A broad peak at 9.02ppm for M1 was observed which is typical short, strong hydrogen bond peak indicating the presence of hydrogen bonding between mixed anion in ionic liquid (Figure.6.1) The results obtained

in the study could be correlated with the appearance of downfield shift of the delocalised proton in aprotic, low polarity solvents (Perrin and Nielson 1997; Kumar et al. 1998; Bica and Rogers 2010). The synthesized benzalkonium based mixed anion ILs have the form and appearance of colourless viscous liquids to clear yellow viscous liquids at room temperature (Table 6.2).

A Similar broad peak was observed in all benzalkonium based mixed anion ILs.

Table 6.1 ^{1}H NMR data of M1 (Benzyltriethylamonium-ibuprofenate salicylic acid)

^{1}H NMR data of M1 in $CDCl_3$ δ ppm (Benzyltriethylamonium-ibuprofenate salicylic acid)	
Benzyltriethylamonium cation	ibuprofen salicylic acid anion
1.16 - 1.28 (t, 9 H), 2.99 - 3.11 (q, 6 H), 4.26 (s, 2 H), 7.19 - 7.31 (m, 5 H)	0.88 (d, 6 H), 1.43 (d, 3 H), 1.74 - 1.87 (m, 1 H) , 2.40 (d, 2 H), 3.69 (q, 1H), 6.65 – 6.77 (t, 1 H), 6.77 – 6.87 (d, 1H), 6.98 – 7.09 (d, 2 H) 7.33 – 7.45 (d, 2H) 7.80 – 7.91 (dd, 1 H), 9.02 (br. S., 1H)

br – broadened singlet, s-singlet, d- doublet, dd-double doublet, t- triplet, q-quartet, m-multiples

NMR and FTIR characterization data of IL with mixed anions

M1 (Benzyltriethylammonium-ibuprofenate salicylic acid)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.88 (d, 6 H), 1.16 - 1.28 (t, 9 H), 1.43 (d, 3 H), 1.74 - 1.87 (m, 1 H), 2.40 (d, 2 H), 2.99 - 3.11 (q, 6 H), 3.69 (q, 1 H), 4.26 (s, 2 H), 6.65 - 6.77 (t, 1 H), 6.77 - 6.87 (d, 1 H), 6.98 - 7.09 (d, 2 H), 7.19 - 7.31 (m, 5 H), 7.33 - 7.45 (d, 2 H), 7.80 - 7.91 (dd, 1 H), 9.02 (br. s., 1 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 7.83, 18.72, 22.40, 30.20, 45.04, 45.41, 52.57, 60.76, 76.75, 77.06, 77.38, 116.46, 117.77, 126.64, 127.42, 129.11, 129.52, 130.80, 130.85, 132.14, 132.98, 138.62, 140.11, 161.97

IR (v_{max} cm^{-1}): 2954, 2931, 2848, 1718, 1655, 1625, 1591, 1512, 1456, 1383, 1214, 754

M2 (Benzyltriethylammonium-salicylcate ibuprofen)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.87 (d, 6 H), 1.15 (t, 9 H), 1.41 (d, 3 H), 1.80 (m, 1 H), 2.38 (d, 2 H), 2.97 (q, 6 H, 3.68 (q, 1 H), 4.18 (s, 2 H), 6.62 - 6.73 (t, 1 H), 6.78 (d, 1 H), 7.00 (d, 2 H), 7.16 - 7.28 (m, 5 H), 7.31 - 7.44 (d, 2 H), 7.85 (dd, 1 H), 11.88 - 12.00 (br, 1 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 7.67, 18.84, 22.36, 22.38, 30.18, 45.02, 45.62, 52.45, 60.57, 76.76, 77.07, 77.39, 116.34, 117.54, 118.92, 126.65, 127.43, 129.02, 129.45, 130.74, 132.09, 132.63, 139.01, 139.90, 162.01, 173.99, 177.83

IR (v_{max} cm^{-1}): 3411, 2954, 2929, 2848, 1708, 1625, 1576, 1456, 1329, 1214, 856.3

M3 (Benzyldimethylhexadecylammonium-ibuprofenate salicylic acid)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.84 - 0.96 (d, 6 H), 1.20 - 1.33 (m, 26 H), 1.50 (d, 3 H), 1.60 - 1.72 (m, 2 H), 1.84 (m, 1 H), 2.43 (d, 2 H), 3.02 (s, 6 H), 3.11 - 3.23 (m, 2 H), 3.70 - 3.79 (q, 1 H), 4.53 (s, 2 H), 6.72 - 6.85 (t, 1 H), 6.85 - 6.96 (d, 1 H), 7.01 - 7.13 (d, 2 H), 7.23 - 7.35 (d, 2 H), 7.37 - 7.49 (m, 5 H), 7.95 (dd, 1 H), 9.27 - 9.49 (br, s, 1 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 14.14, 18.56, 22.40, 22.71, 22.79, 26.20, 29.16, 29.38, 29.48, 29.62, 29.68, 29.71, 29.73, 29.73, 30.19, 31.94, 45.04, 45.33, 49.83, 63.97, 68.11, 76.74, 77.05, 77.37, 116.67, 118.12, 126.82, 127.41, 129.19, 129.34, 130.84, 130.87, 132.83, 133.49, 138.31, 140.28, 161.91

IR (v_{max} cm^{-1}): 3422, 2954, 2923, 2853, 1715, 1620, 1592, 1484, 1457, 1252, 778.8

M4 (Benzyldimethylhexadecylammonium-salicylcate ibuprofen)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.81 - 0.92 (d, 6 H), 1.10 - 1.26 (m, 26 H), 1.44 (d, 3 H), 1.51 - 1.63 (m, 2 H), 1.79 (m, 1 H), 2.38 (d, 2 H), 2.92 (s, 6 H), 3.03 - 3.14 (m, 2 H), 3.71 (q, 1 H), 4.45 (s, 2 H), 6.67 - 6.79 (t, 1 H), 6.84 (d, 1 H), 6.94 - 7.05 (d, 2 H), 7.19 - 7.28 (d, 2 H), 7.33 - 7.45 (m, 5 H), 7.92 (dd, 1 H), 12.01- 12.06 (m, 1 H)

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 14.12, 18.76, 22.39, 22.69, 22.71, 22.73, 26.20, 29.16, 29.37, 29.39, 29.48, 29.62, 29.67, 29.72, 30.18, 31.93, 45.05, 45.69, 49.69, 63.72, 67.91, 76.73, 77.05, 77.37, 116.43, 117.63, 119.08, 127.02, 127.47, 129.06, 129.26, 130.74, 132.68, 132.87, 138.99, 139.94, 162.09, 174.16, 177.97

IR (v_{max} cm^{-1}): 3471, 2954, 2924, 2853, 1710, 1624, 1577, 1458, 1216, 856.7, 702.6

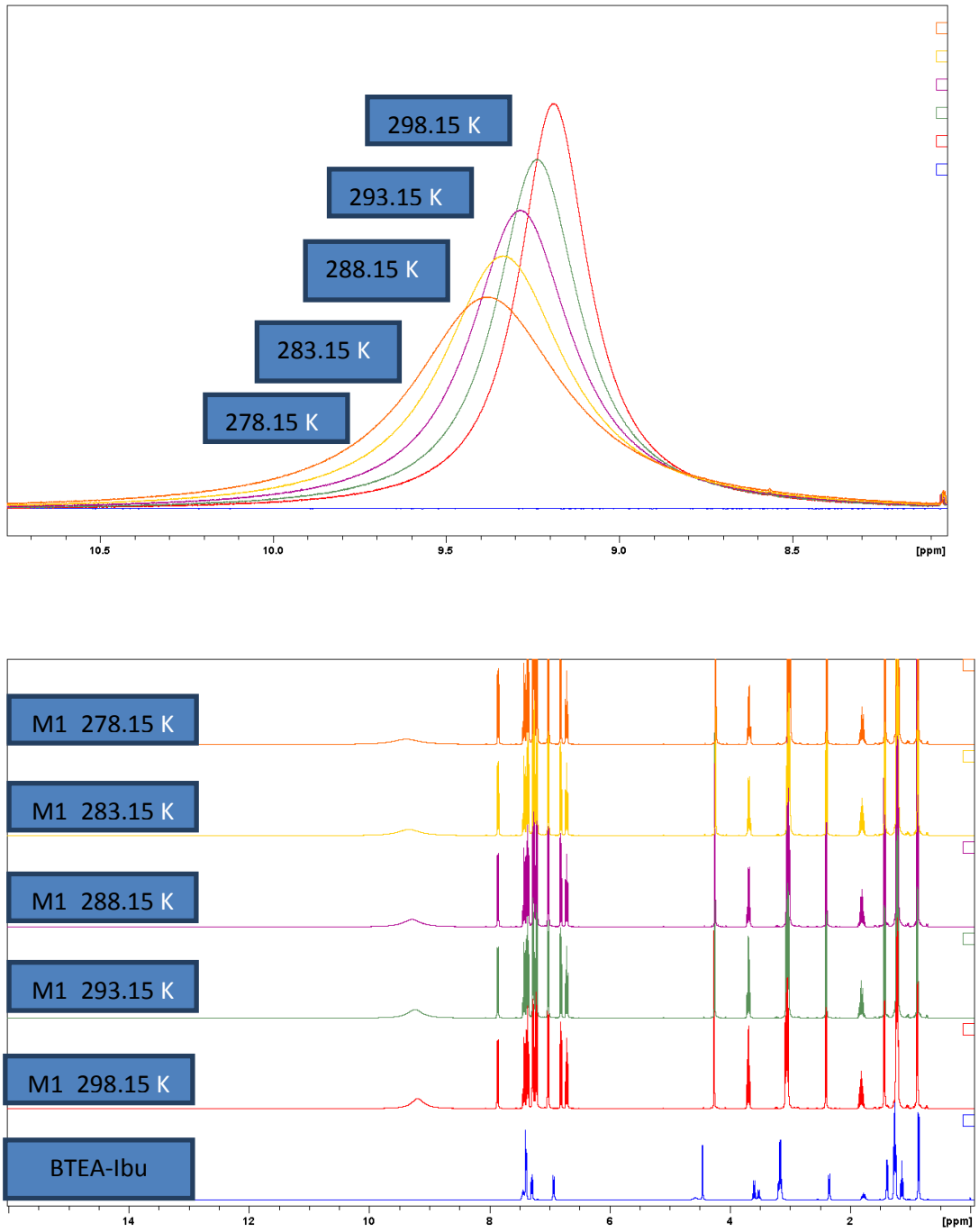


Figure 6.1 ^{1}H NMR spectra of [BTEA-Ibu] (bottom) and M1 (top) at various temperature

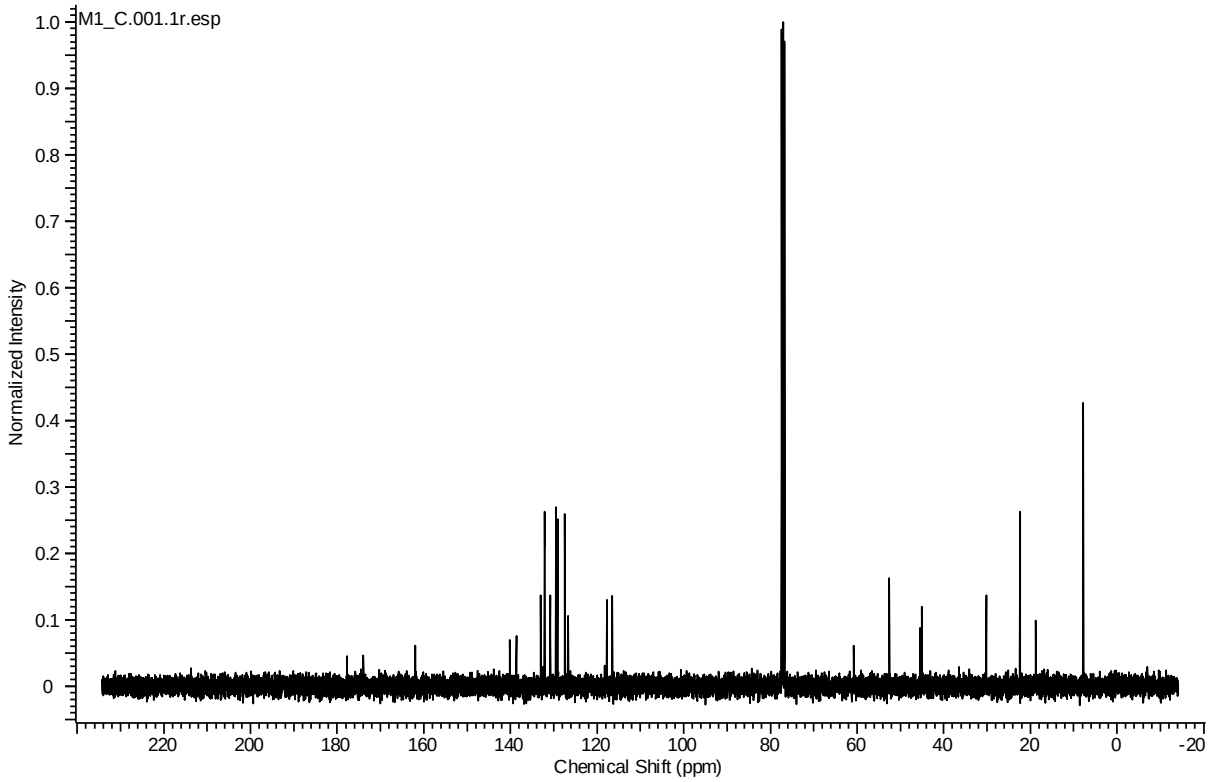


Figure 6.2 ^{13}C spectra of M1

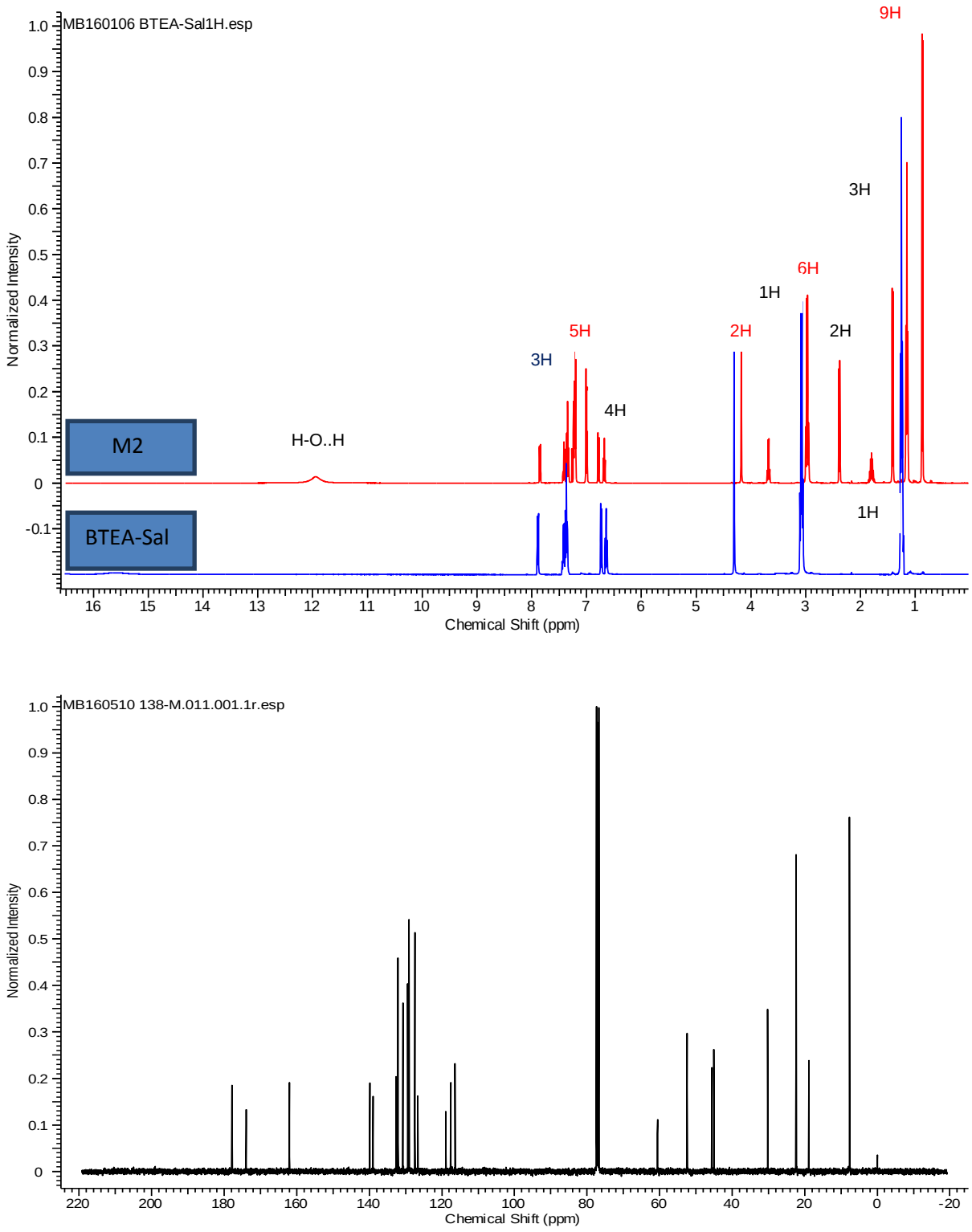


Figure 6.3 ^{1}H and ^{13}C NMR spectra of M2

[BTEA], [Salicylate] and [Ibuprofen] protons are represented in red, blue and black colours respectively

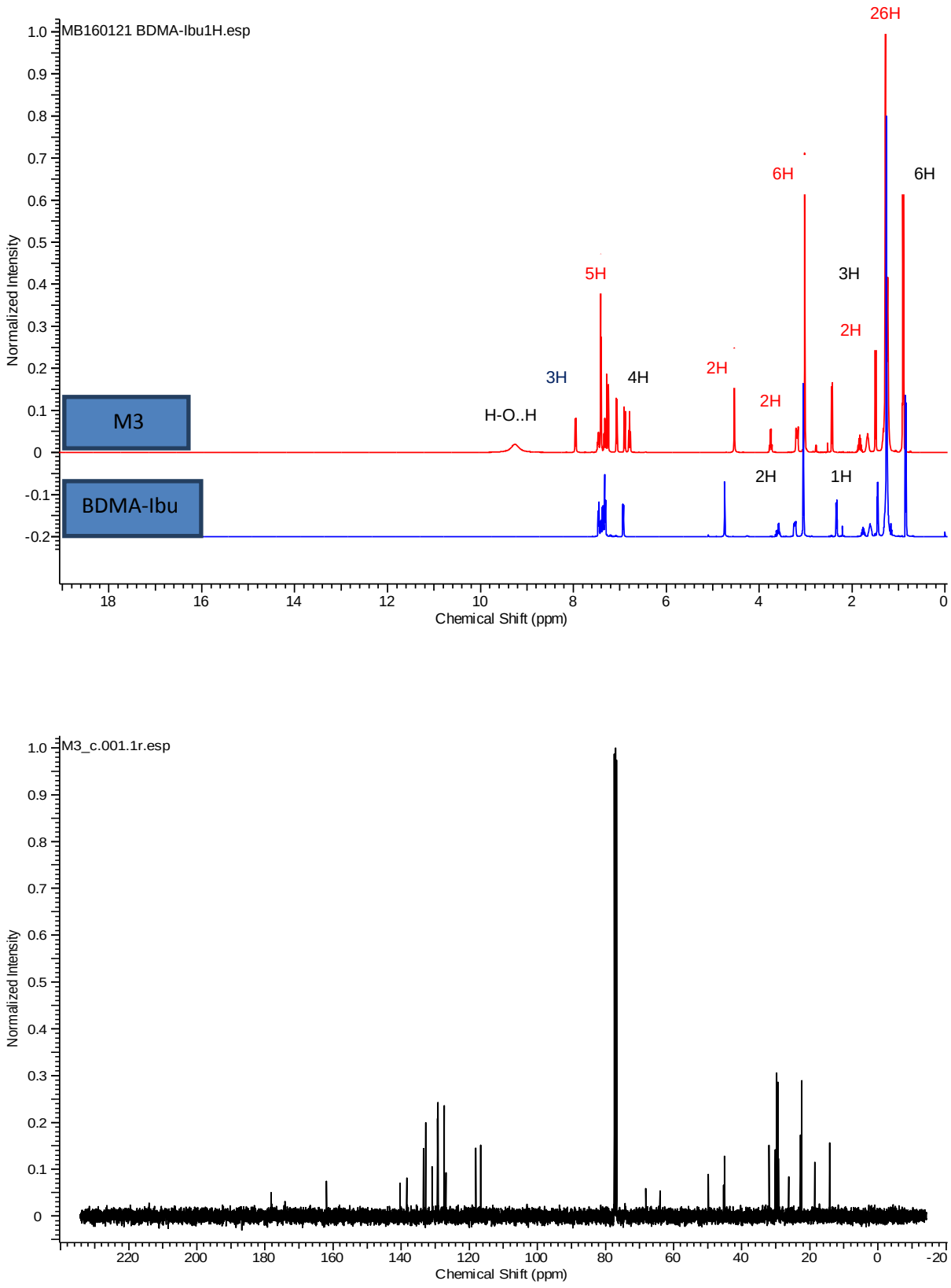


Figure 6.4 ^{1}H and ^{13}C NMR spectra of M3

[BDMA], [Salicylate] and [Ibuprofen] protons are represented in red, blue and black colours respectively

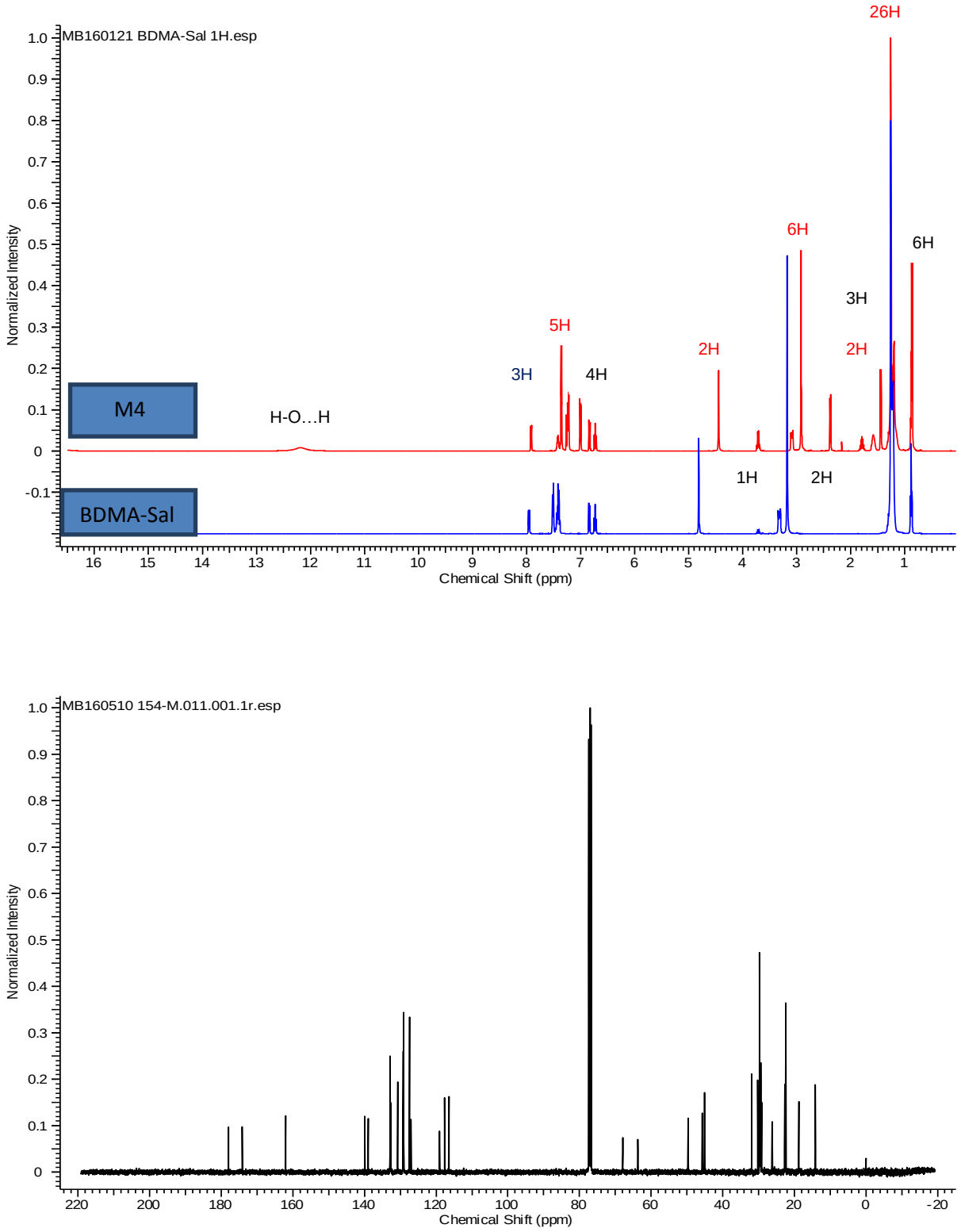


Figure 6.5 ^{1}H and ^{13}C NMR spectra of M4

[BDMA], [Salicylate] and [Ibuprofen] protons are represented in red, blue and black colours respectively

6.2.2 Thermal Behaviour

The thermal properties of the benzalkonium based mixed anion ILs were studied using TGA and DSC. Table.6.2 shows the thermal stability of studied benzalkonium based mixed anion ILs ranging from 180.33 ˚C to 185.07 ˚C. All the benzalkonium based mixed anion ILs are viscous liquids at room temperature and thermogravimetric analysis showed a single decomposition step for all studied ILs (Figure 6.6 - 6.9).

The thermal decomposition temperature was found to be in the order of M2 > M1. The impact of interactions between counterions can be observed by difference in thermal stability of ILs with mixed anions. It could be observed from the trend that thermal stability of the ILs with mixed anions decreases with increase in anion size of the salt (asymmety), the negative charge is spread over the large volume of anion resulting in much weaker interactions between the counterions (Leys et al. 2008). The same pattern was observed for M4 > M3. However, when the decomposition temeratures of ILs with mixed anions were compared on the basis of presence of cation, then order of thermal stability would be in the order of M3 > M1 and M4 > M2. The increase in decomposition temperature could be attributed due to increase in interparticle forces (Van der Waal forces) which arises with increase in alkyl chain length (Canongia Lopes and Padua 2006; Chancelier et al. 2016). Although little difference in thermal decomposition temperature was observed among benzalkonium based mixed anion ILs.

DSC thermograms show the glass transition temperatures for all of the synthesized benzalkonium based mixed anion ILs in the following order - 42.59 (M1), -35.99 (M2), -33.52 (M3) and

-48.30 and -30.79 for (M4) (Table 6.2). The presence of glass transition temperature provides significant evidence that the material is amorphous. The DSC thermogram of M3 (Figure 6.8) shows peaks corresponding melting temperature (Tm) of -12.67 °C and crystallization temperature (Tc) which is found at -28.82 °C during cooling step, where molecules embrace more ordered structure after releasing energy. Please do refer table 3.1 for M1, M2, M3 and M4 mixed anion ILs.

TGA and DSC profiles of benzalkonium based mixed anion ILs

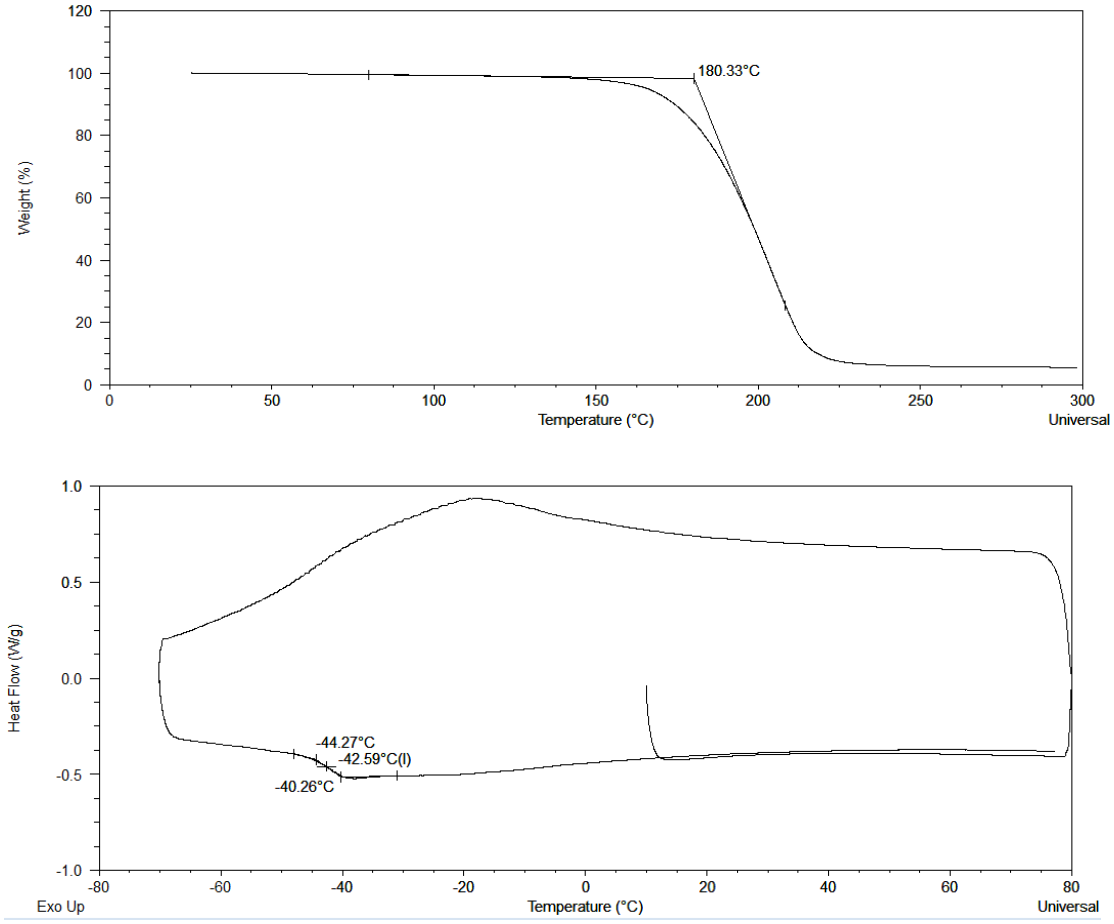


Figure 6.6 TGA and DSC profiles of M1

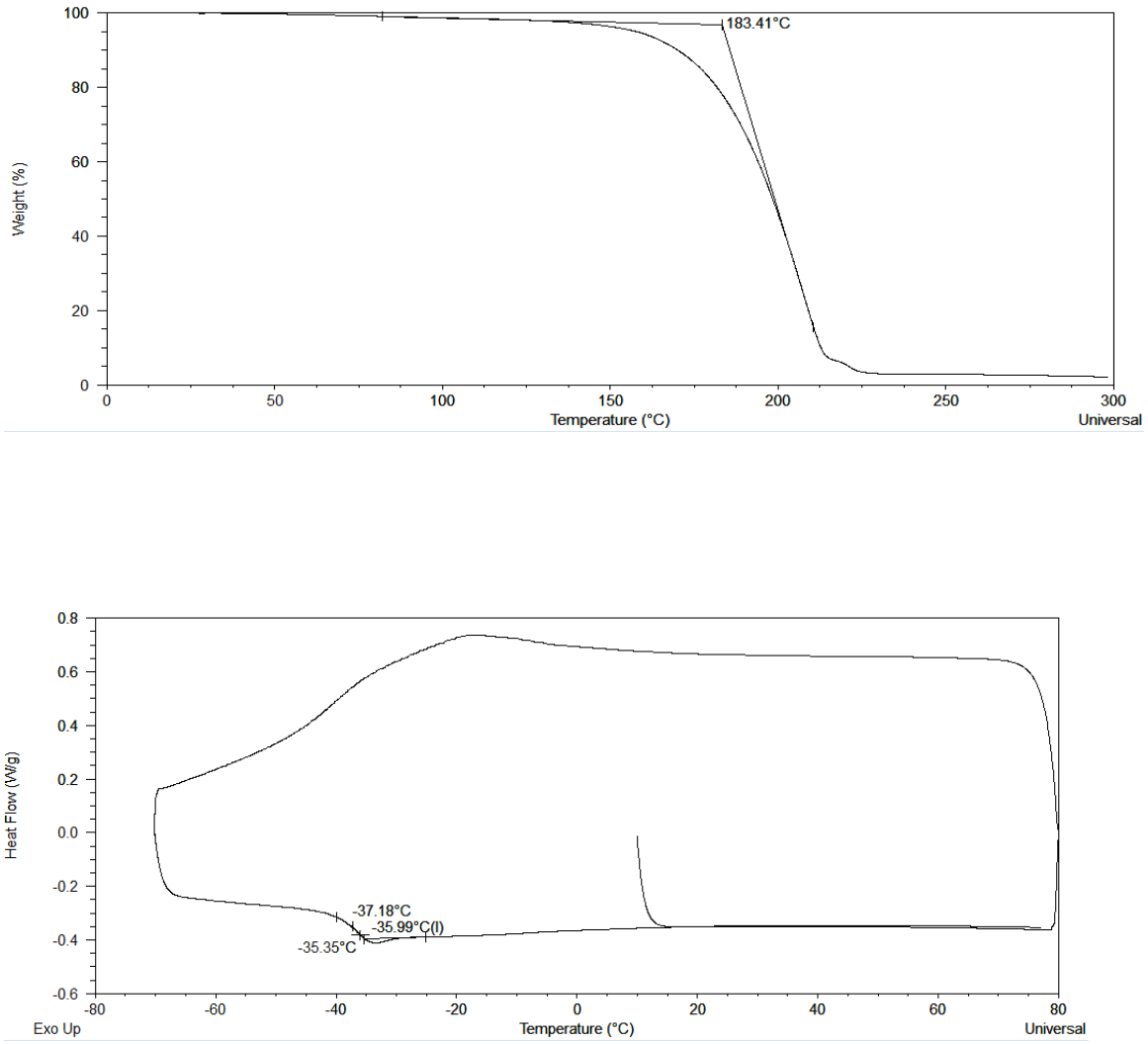
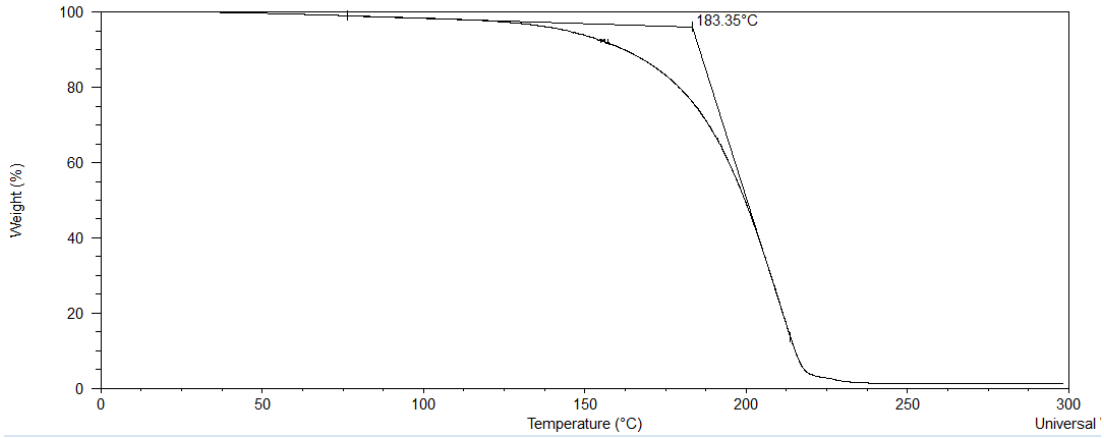


Figure 6.7 TGA and DSC profile of M2



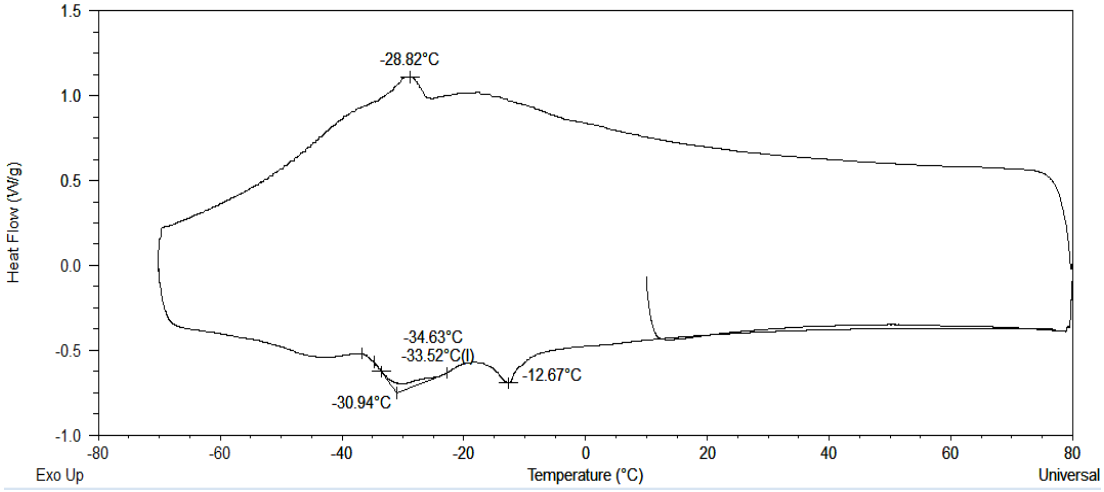
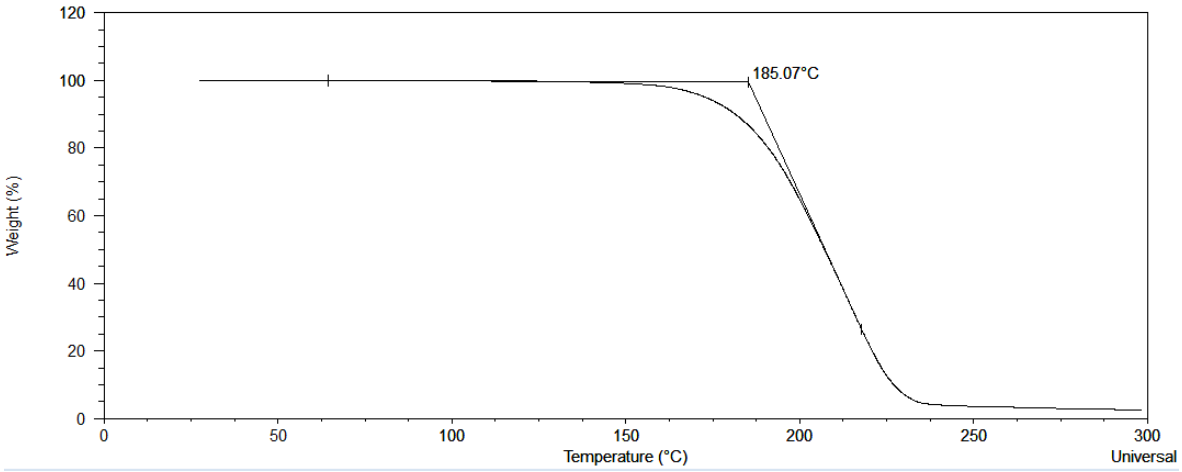


Figure 6.8 TGA and DSC profile of M3



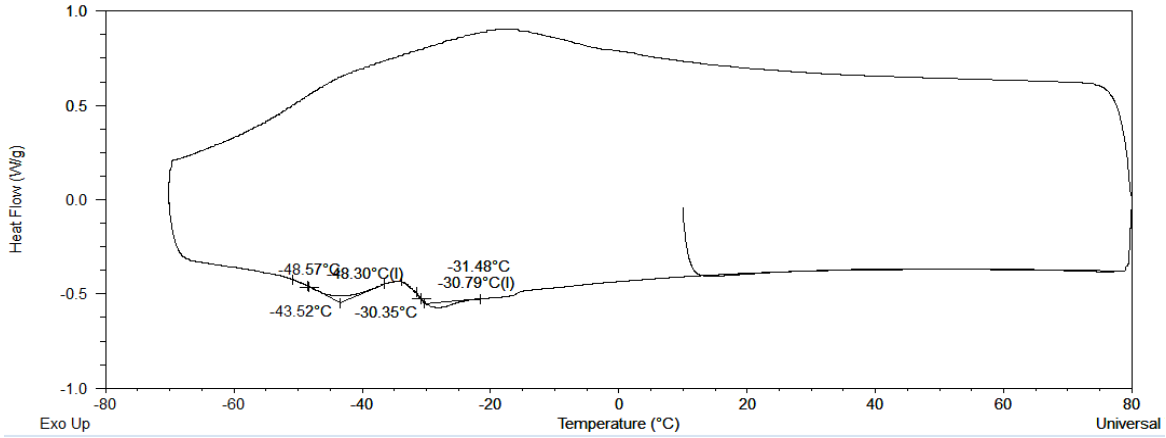


Figure 6.9 TGA and DSC profile of M4

6.2.3 Octanol-water partition coefficient

Lipophilicity of the molecule is the vital element which is described by the octanol-water partition coefficient, used to correlate the absorption and transport properties of the active pharmaceutical ingredient in the human body (Pinto et al. 2013). In this study the lipophilicity of the benzalkonium based mixed anion ILs were assessed via experimental determination of octanol-water partition coefficient. The results of the study are summarised in Table.6.2. As can be seen, in Figure.6.10 the octanol-water partition coefficient for M3 and M4 are higher than those of M2 and M1 ILs with mixed anions. The high value obtained for octanol-water partition coefficient can be attributed to the increase in the molecular weight and strong Van der Waals interactions which increases with increase in the long alkyl chains they have (Domańska et al. 2003; Wu et al. 2003; Padró et al. 2014) From experimental results obtained for M1 and M2, the octanol-water partition coefficient was found to be higher for M1. This observation could be correlated to the hydrophobic nature of ibuprofen anion, due to its bigger size to charge ratio as compared to the salicylate anion favouring dispersive interactions with organic molecules (Padró et al. 2014). However due to small salicylate anion compared with ibuprofen anion exhibits high ionic density leading to strong electrostatic interactions between cation and anion (Ingram et al. 2011), and which might also allow the formation of hydrogen bonding with nearby organic molecule. Same pattern was observed with M3 and M4 benzalkonium based mixed anion ILs.

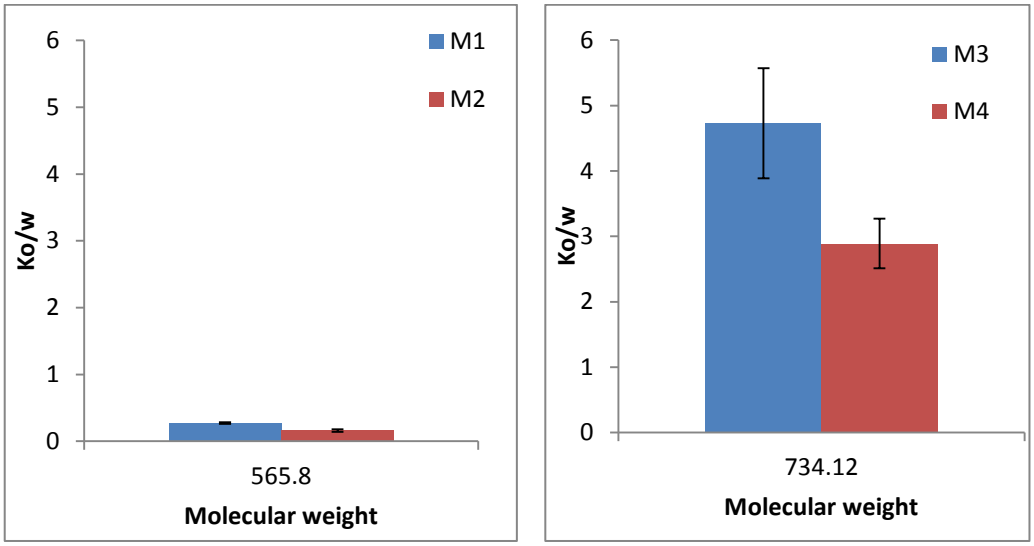


Figure 6.10 Octanol-water coefficient of benzalkonium based mixed anion ILs (Error bars - standard deviation)

Table 6.2 Physicochemical properties of benzalkonium based mixed anion ILs

IL with mixed anions	Ko/w	Molecular weight	Tg(°C)	Tc(°C)	Tm(°C)	Td(°C)	Physical state
M1	0.27(±0.019)	565.80	-42.59	-	-	180.33	Colourless Viscous liquid
M2	0.16(±0.012)	565.80	-35.99	-	-	183.41	Yellow viscous liquid
M3	4.79(±1.24)	734.12	-33.52	-28.82	-12.67	183.35	Colourless Viscous liquid
M4	2.89(±0.38)	734.12	-48.30, -30.79	-	-	185.07	Yellow viscous liquid

Tg – glass transition temperature ; Tc – crystallization temperature ; Tm – melting point ; Td – decopmposition temperature

6.2.4 Electrical conductivity

Ionic liquids are generally composed of charged carrier ion therefore ILs are expected to have large electrical conductivity window. The electrical conductivity of ionic liquids is different from solutions having dissociated ions or nonpolar liquids. This leads towards interest in measurement of the electrical conductivity of synthesized ILs at different concentrations in water (Widegren et al. 2005).

In order to investigate the influence of interactions between counterions on the conductivity, the electrical conductivity profiles of aqueous solutions of M1, M2, M3, and M4 benzalkonium based mixed anion ILs were studied at the highest concentration range. The results of conductivity are shown Figure. 6.11. There was no significant difference observed for the room temperature conductivity values of M1 and M2 ILs with mixed anions. It can be observed from the figure that electrical conductivity data shows the trend of salt in aqueous solution where the conductivity increase with increase in concentration of ILs. Aqueous solution of ILs behaves similar way to concentrated salt solutions. The conductivity of ILs aqueous solution increases due to the presence of water-rich regions. The dissociation constant of ILs was increased with increase in the amount of water (Tshibangu et al. 2011).

As described earlier, to investigate the conductivity profiles of M3, M4 benzalkonium based mixed anion ILs the aqueous solutions were prepared at highest concentration. But due to hydrophobic nature, increased molecular volume and viscosity M3, M4 ILs with mixed anions failed to give clear aqueous solutions.

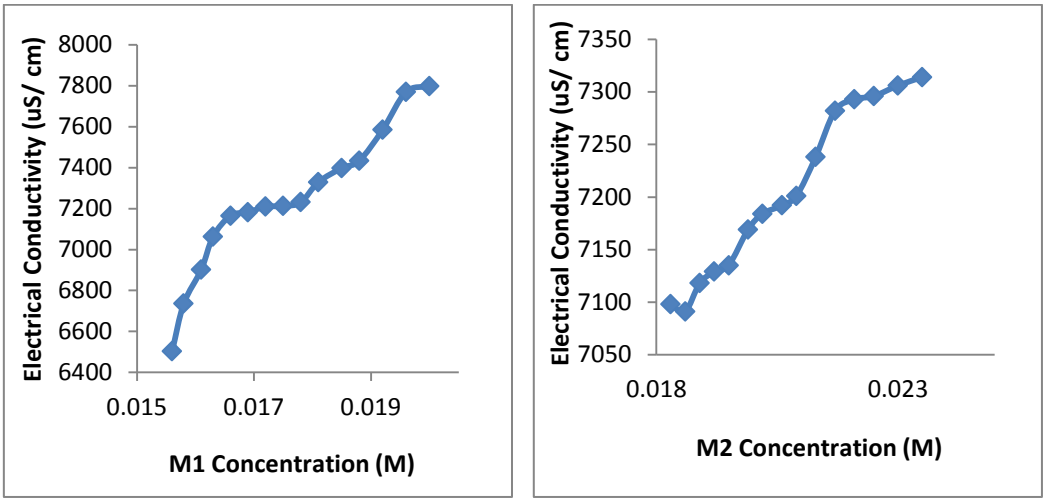


Figure 6.11 Electrical conductivity profiles of benzalkonium based mixed anion ILs

6.2.5 Permeation studies

Neat benzalkonium based mixed anion IL was applied on artificial membrane and assessment of the drug permeation via artificial membrane was studied. The reason behind the direct application of neat IL on the skin excludes the need of other excipients which are generally used to solubilize the drug molecules.

6.2.5.1 Evaluation of ibuprofen and salicylic acid permeation through membrane in different forms

Figure.6.12 summarises the permeation rates of ibuprofen and salicylic acid in different forms through the membrane over time from M1 and M2 (as neat IL). As can be seen that transport rate of ibuprofen from M1 and M2 via hydrophilic membrane is much slower and no significant difference observed in the permeation rates of neutral ibuprofen which is hydrogen bonded (M2) and ibuprofen in the form of IL salt (M1). The data suggest that ibuprofen

transport through the membrane in almost identical manner in both forms. Salicylic acid permeates the membrane more rapidly, achieving saturation level within 6-7 hour in both forms from M1 and M2. The transport of salicylic acid in the form of IL salt (M2) and salicylic acid as neutral molecule which is hydrogen bonded (M1) found to permeate at the same rates. Thus it appears, salicylic acid and ibuprofen (ionized or hydrogen bonded neutral form) are associated strongly via ionic/hydrogen bonding interaction and remain intact as single entity which allows the passage of individual API simultaneously through membrane.

On the other hand, permeation of ibuprofen and salicylic acid from M1 and M2 through hydrophobic membrane shows different behaviour. The transport rate of ibuprofen in the form of IL salt from M1 was found to be higher compared to the hydrogen bonded neutral ibuprofen from M2. The results obtained are found to be in accordance with the results reported by *Sarveiya* et al.(2004)where ion-pairing was responsible for achieving better permeation rate through a polydimethylsiloxane membrane (Sarveiya et al. 2004). While in case of salicylic acid, the permeation rate was found to be higher when salicylic acid was in hydrogen bonded neutral form (M1) than in the form of IL salt (M2), the results obtained here could be correlated to studies performed by Stoimenovski et al where they observed acidic molecule transport easily over the IL salt form (Stoimenovski and MacFarlane 2011). The difference in the permeation rates of ibuprofen and salicylic acid from neat IL through hydrophobic membrane could be attributed to the nature of membrane, molecular mass and solubility of the IL.

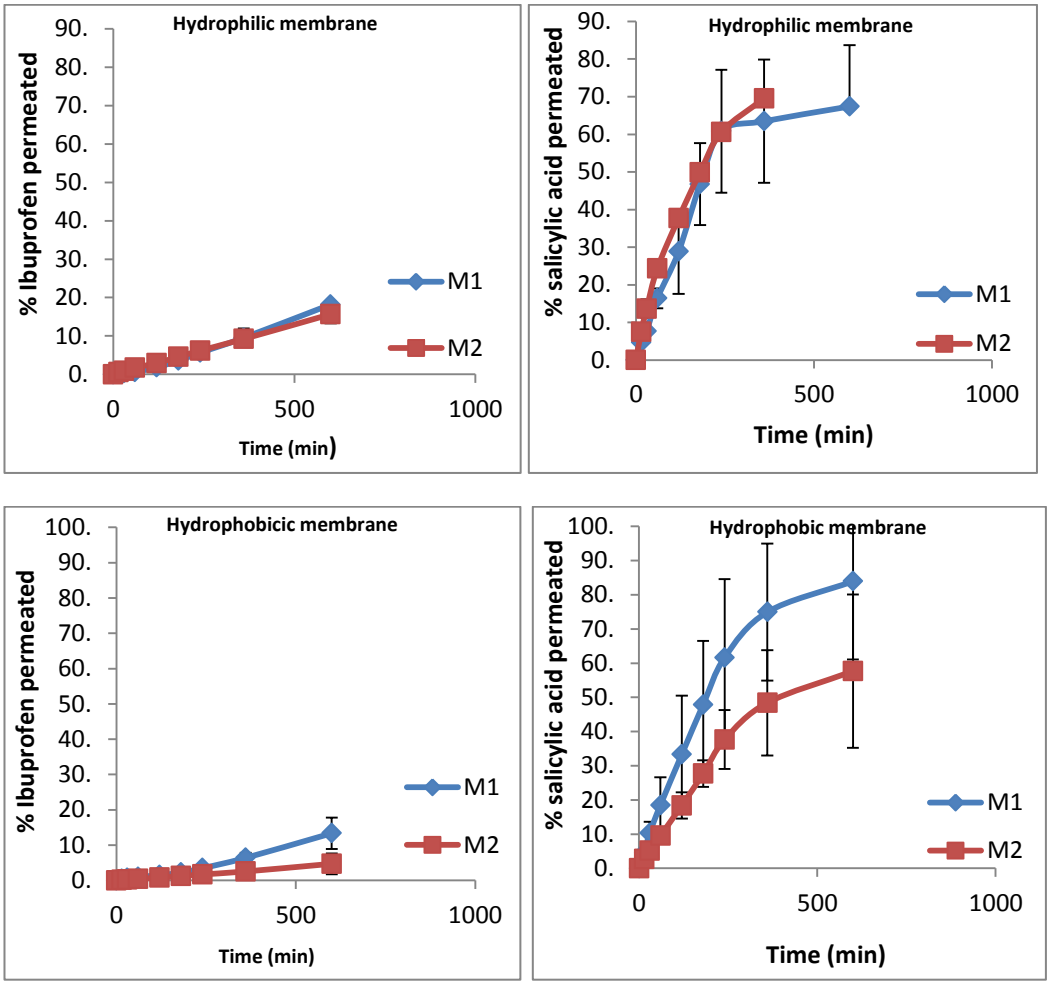


Figure 6.12 Permeation of % ibuprofen and salicylic acid from M1 and M2 through hydrophilic and hydrophobic membranes (Error bars - standard deviation)

The ibuprofen and salicylic acid permeation in different forms through the membrane over time from M3 and M4 (as neat IL) is shown in Figure.6.13. The permeation rate of ibuprofen and salicylic acid in different forms from hydrophilic membrane was found to be significantly different from what we observe in case of M1 and M2. The permeation rate of ibuprofen in hydrogen bonded neutral form (M4) was found to higher compared to ibuprofen in the form of IL salt (M3) whereas in case of salicylic acid the permeation profile is just reverse and was found to be higher in the form of IL salt (M4) compared to salicylic acid in neutral form hydrogen bonded molecule (M3). The results obtained could be attributed to the difference in the interactions between cation and anions and to their octanol water partition coefficient values. The increase in the anion size (keeping cation constant) leads to decrease in ionic interactions due to delocalisation of charge (Freire et al. 2007; Leys et al. 2008; Alves et al. 2013), and finally transport properties. The increased ionic interaction between cation and anion (M4) is responsible to form more strong hydrogen bonding with ibuprofen. The increased ionic interaction and hydrogen bonding in M4 leading to generate single entity which finally increased permeation rate compared to corresponding M3. Similar kind of pattern was found for permeation rate of ibuprofen and salicylic acid through hydrophobic membrane but in significantly slow rate compared to hydrophilic membrane.

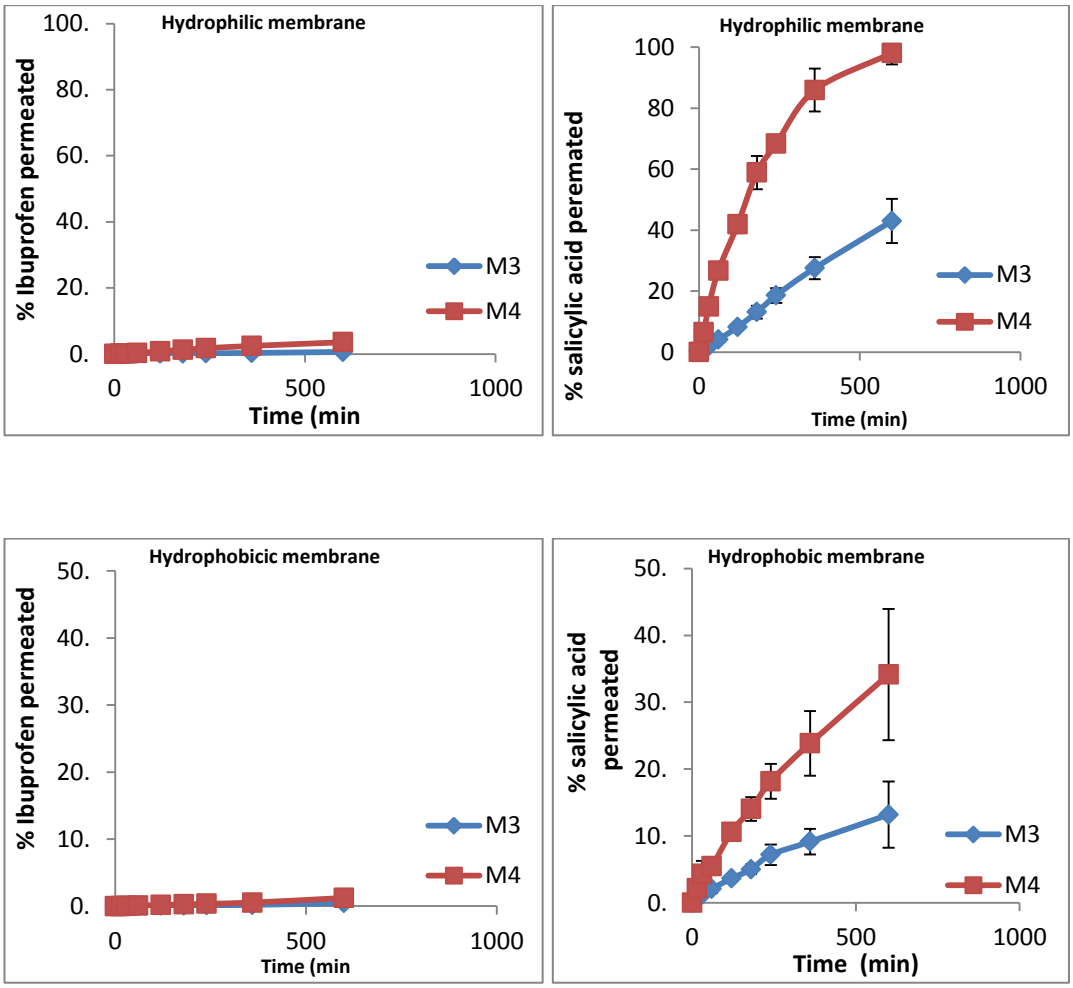


Figure 6.13 Permeation of % ibuprofen and salicylic acid from M3 and M4 through hydrophilic and hydrophobic membranes (Error bars - standard deviation)

6.2.5.2 Effect of cation on permeation of ibuprofen and salicylic acid (same form) through membrane

Comparing the permeation of ibuprofen and salicylic acid via different membrane on the basis of alkyl chain length associated with cation suggest that the permeation of ibuprofen and salicylic acid decrease with increase in the alkyl chain length on the cation. The obtained results could be correlated with the increase in the molecular mass leading to decrease in diffusion. The Van der Waal interaction due to alkyl chain length of cation helps to reduce

the amount of permeation (Tayar et al. 1991; Pugh et al. 1996). Scheuplein et al. also suggested that small molecules display better and faster permeation profiles compared to larger molecules (Scheuplein et al. 1969). The only promising candidate which appears to have higher permeation through hydrophilic membrane with increase in the alkyl chain length on cation is salicylic acid (M4). The permeation of salicylic acid in the form of salt (M4) with cation having long alkyl chain was found to be dominating over other form, which could be attributed to the increase in lipophilicity of the drug salt. This increases the solubility of drug salt within lipid domains of the stratum corneum and finally improves the membrane permeability (Flynn and Yalkowsky 1972). The ibuprofen and salicylic acid permeation profiles through membranes can be seen in Figure 6.14.

6.2.5.3 Effect of cation on permeation of ibuprofen and salicylic acid (different form) through membrane

A similar pattern was observed on permeation of ibuprofen and salicylic acid (different form) through membrane (Figure 6.15).

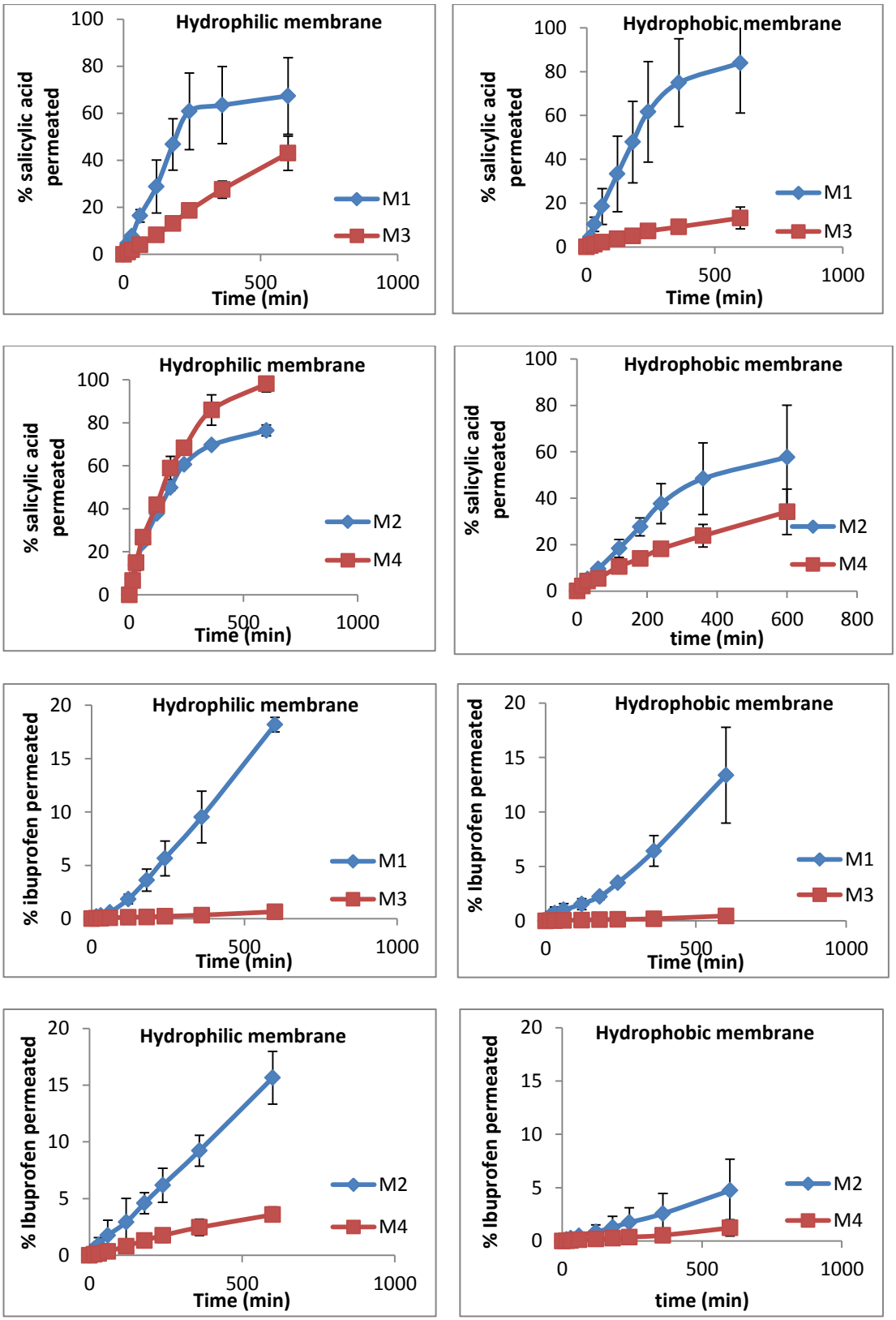


Figure 6.14 Effect of cation on permeation of % ibuprofen and salicylic acid (same form) through membrane (Error bars - standard deviation

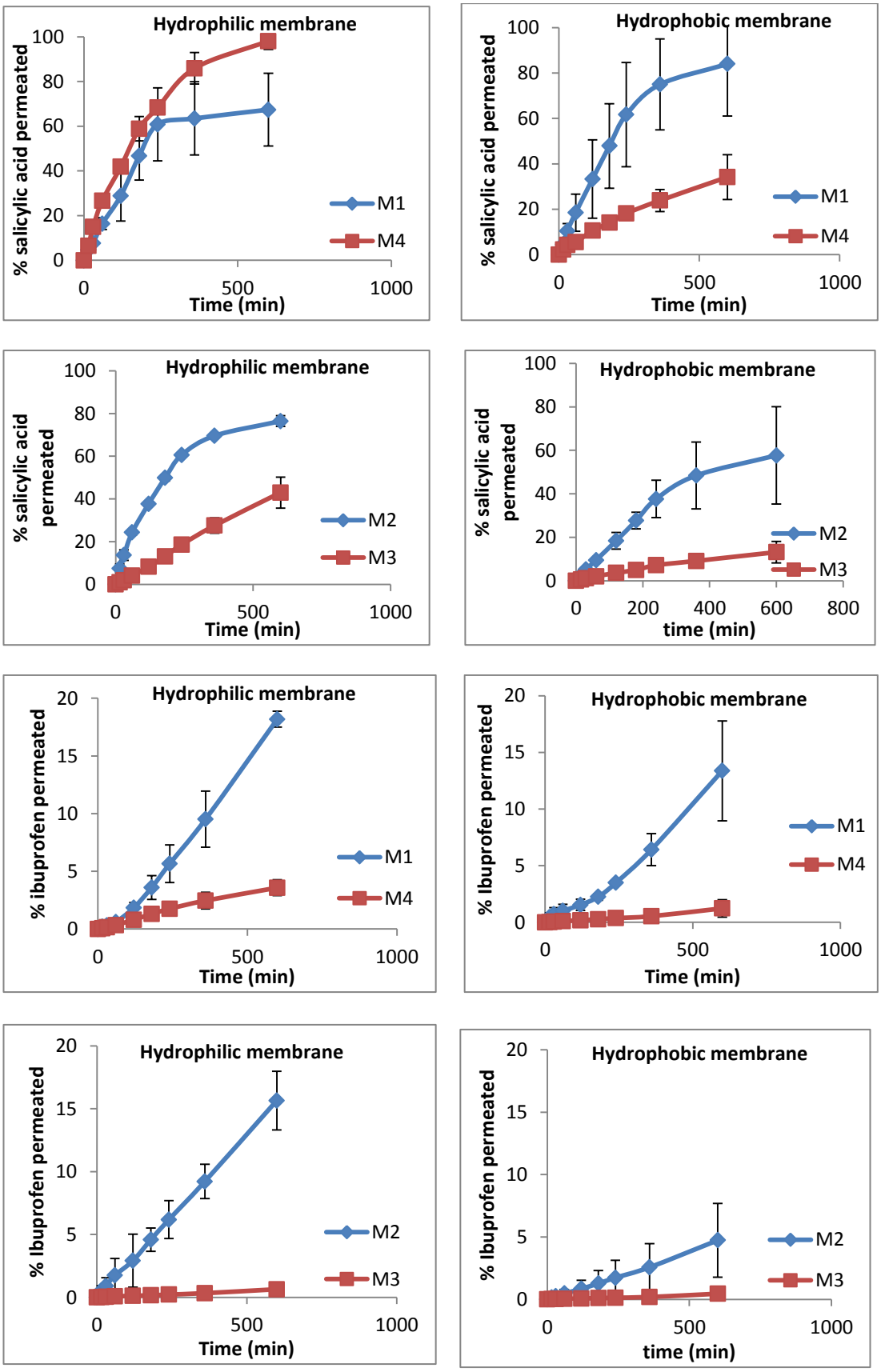


Figure 6.15 Effect of cation on permeation of % ibuprofen and salicylic acid (different form) through membrane (Error bars - standard deviation)

6.3 Conclusion

Benzalkonium based mixed anion ILs were composed of benzalkonium chloride, ibuprofen and salicylic acid which are pharmaceutically approved molecules. The interactions exist in these ILs not only have huge impact on the physicochemical properties but also provides the potential feasibility for these neat IL formulation to penetrate through membrane more effectively. Although *ex-vivo* studies are required to support the data generated. Thus, benzalkonium based mixed anion ILs are effective and possible promising formulation for the topical drug delivery.

Chapter 7

Formulation containing IL hydrogel

7.1 Introduction

The objective of this chapter is to investigate the interactions between various components of the formulation and to demonstrate in-situ formation of ibuprofen IL during formulation for topical treatment. For this purpose, Ibuprofen has been selected as a model drug in its acidic form, alkanolamine (diisopropanolamine, triisopropanolamine, triethanolamine) as base and carbopol 974 was used as gelling agent. The synthesised alkanolamine-ibuprofen IL was characterised by NMR and IR spectroscopy, DSC and TGA.Ibuprofen-IL hydrogels are prepared by incorporating diisopropanolamine-ibuprofen (DIPA-Ibu) IL into 1% aqueous carbopol dispersion, which was characterised by FTIR spectroscopy. In vitro permeation and release studies were also conducted.

The commonly used Non-Steroidal Anti-inflammatory Drugs (NSAIDs) such as ibuprofen, piroxicam and diclofenac are poorly soluble molecules which are formulated in the form of topical gels, creams and patches. Though there are number of gel compositions reported the most commonly commercially used gel formulations contain an acrylic acid polymer as gelling agent. Carbopol 974P (Carbopol) is a FDA approved water swellable high molecular weight acrylic acid polymer toxicologically more preferred over other alternatives of Carbopol polymers (Bonacucina et al. 2004). Carbopol upon neutralisation forms a gel with excellent rheological and bioadhesive strength properties in less than 2-3 % w/v concentration (MORIMOTO et al. 1985; Witschi and Mrsny 1999; Callens et al. 2003; Najafabadi et al. 2004). Low

molecular weight amines and alkanolamines such as triethanolamine, triisopropanolamine, and diisopropanolamine are commonly used for neutralisation of Carbopol. The carboxylic groups of the Carbopol 974P molecules would be uncharged at low pH and display weak interactions and found to be in coiled state. The carboxylic group of the carbopol 974P is converted into carboxylates at nearly neutral and alkaline pH and the repulsion between the negatively charged carboxylates is responsible for the expansion and finally the formation of hydrogel (Park and Robinson 1985; Handbook 1993).

The conventional dosage form such as preformed gels do not remain for the long time and required frequent dosing. The in-situ gel system approach using carbopol 974P not only combines the advantage of gel formation but in addition to that an accurate amount of dose can be applied on to the administration site. These formulation remains in solution state before application to the administration site and transformed into gel state after administration. Moreover these polymers display the ability to tailor the drug release from gels (Valenta 2005). The synthesis of ionogels encapsulating IL (1-methyl-3-butylimidazolium-ibuprofen) in porous functionalised silica has been reported in literature (Viau et al. 2010).

Recently, there are many reports about design of Active Pharmaceutical Ingredient (API) – ILs to improve performance of the API by tailoring physicochemical properties such as solubility, lipophilicity, melting point and viscosity. API – ILs have also been used to incorporate dual activity for example lidocaine-docusate improved efficacy of lidocaine as local anaesthetic along with emollient effect of docusate while docusate as a

counter ion was effective in avoiding polymorphic transformation of ranitidine. There is growing focus on exploring use of API – ILs to achieve improved transport of drugs across the lipophilic barriers. Miwa et al. reported significant improvement in the solubility and transdermal permeation of etodolac when used as etodolac-lidocaine IL. Wang et al. identified strong charge assisted hydrogen bond complex formation between ibuprofen and lidocaine. This complex was termed as liquid – cocrystal and the pair is transported across the membrane as a complex (Wang et al. 2014). Sarveiya et al. also showed association between the two components of the IL. The lipophilic drug and counterion neutral aggregates penetrate the dermal layers instead of drug in the ionic form. Though there are advantages and encouraging permeation data has been reported, a clinical trial transdermal patch (Etoreat) containing etodolac –lidocaine IL has been terminated due to failure to show significant pain relief compared to placebo. As per our knowledge there is no FDA approved ionic liquid formulation in the market.

Ionic liquid based on ibuprofen drug was synthesised in which Ibuprofen was chosen in its acidic form to allow proton transfer. Diisopropanolamine, triisopropanolamine, triethanolamine (aliphatic amine) were carefully chosen as they have been used in cosmetic and pharmaceutical formulations. Alkanolamine-ibuprofen IL was prepared by neutralisation reaction and then the synthesised IL was used to incorporate into 1% aqueous carbopol dispersion to generate hydrogels. These hydrogels were subjected to investigate thepotential of In-situ formation of ibuprofen – IL in topical ibuprofen gel formulationand the influence of diisopropanolamine (present in

diisopropanolamine ibuprofen) on in vitro release and permeation profiles was studied.

7.2 Results and Discussion

7.2.1 Characterisation of diisopropanolamine-ibuprofen ionic liquid

For model ILs, we tried for three ILs namely diisopropanolamine-ibuprofen (DIPA-Ibu), triisopropanolamine-ibuprofen (TIPA-Ibu) and triethanolamine-ibuprofen (TEA-Ibu). The first two were prepared by neutralisation reaction and characterised by ^{1}H NMR, ^{13}C NMR, FTIR but ibuprofen was found to be precipitated in case of triethanolamine-ibuprofen. In order to select best IL candidate for incorporation in carbopol dispersion, we performed dissolution studies and the data suggest diisopropanolamine-ibuprofen as a promising candidate for further studies as its water solubility is far greater than the aqueous solubility of triisopropanolamine-ibuprofen (39.71 mg/ml) and triethanolamine-ibuprofen (35.51 mg/ml) respectively. The solubility data is provided in appendix. Then the ionic liquid diisopropanolamine-ibuprofenate was further carried for next studies and to generate ibuprofen hydrogels using 1% aqueous carbopol dispersion.

^{1}H NMR spectroscopy dataof diisopropanolamine-ibuprofen(DIPA-Ibu) showed the peaks at 0.87 ppm and 6.93-7.23 ppm, which is attributed to the methyl groups and aromatic ring of ibuprofen, while the peaks observed at 1.06 ppm and 2.58-2.86 ppm correspond to methyl and methylene groups of diisopropanolamine respectively (Figure 7.1).This data was further supported by FTIR spectra of diisopropanolamie, ibuprofen and diisopropanolamie-ibuprofen IL (Figure 7.2). The absorption band of the carboxylic group of ibuprofen appeared at 1709 cm^{-1} while a broad band at 3276 cm^{-1} appears for diisopropanolamine which is attributed due to absorption overlap between the amino group and the hydroxyl group of diisopropanolamine. The band

that arises near 2955-2960 cm^{-1} corresponds to the aliphatic groups found in both the aliphatic amines and carboxylic compounds. The absorption band of the carboxylic group of ibuprofen was shifted to 1560 cm^{-1} from 1709 cm^{-1} in the diisopropanolamine-ibuprofen IL. The absorption band near 1560 cm^{-1} was an indication of salt of aliphatic carboxylic acids. The appearance of a new band at 1560 cm^{-1} and disappearance of the band at 3276 cm^{-1} confirms the formation of diisopropanolamine-ibuprofen salt in a uniform colourless liquid at room temperature.

Thus the ^{1}H NMR spectroscopy and FTIR absorption spectrum analysis confirmed the formation of ionic liquid by the neutralisation reaction between diisoropanolamine and ibuprofen. The ionic liquid was composed of equimolar ratios (1/1 mol/mol) of the reactants.^{1}H, ^{13}CNMR and FTIR data of triisopropanolamine-ibuprofen is provided in appendix.

NMR and FTIR characterization data

Diisopropanolamine-ibuprofen:

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (6 H), 1.06 (6H), 1.27 - 1.39 (3 H), 1.75 - 1.87 (1 H), 2.40 (2 H), 2.58 - 2.69 (2 H), 2.74 - 2.86 (2 H), 3.40 - 3.53 (2 H), 3.85 - 3.97 (2 H), 6.93 - 7.05 (2 H), 7.23 (2 H)

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 19.49, 21.01, 21.04, 22.10, 29.66, 44.39, 47.19, 54.66, 54.85, 62.56, 62.70, 127.06, 128.37, 138.28, 141.21, 178.77

IR (ν_{max} cm^{-1}): 3188, 2957, 2869, 2846, 1560, 1457, 1307, 1255, 1141, 1064, 1020, 928, 849

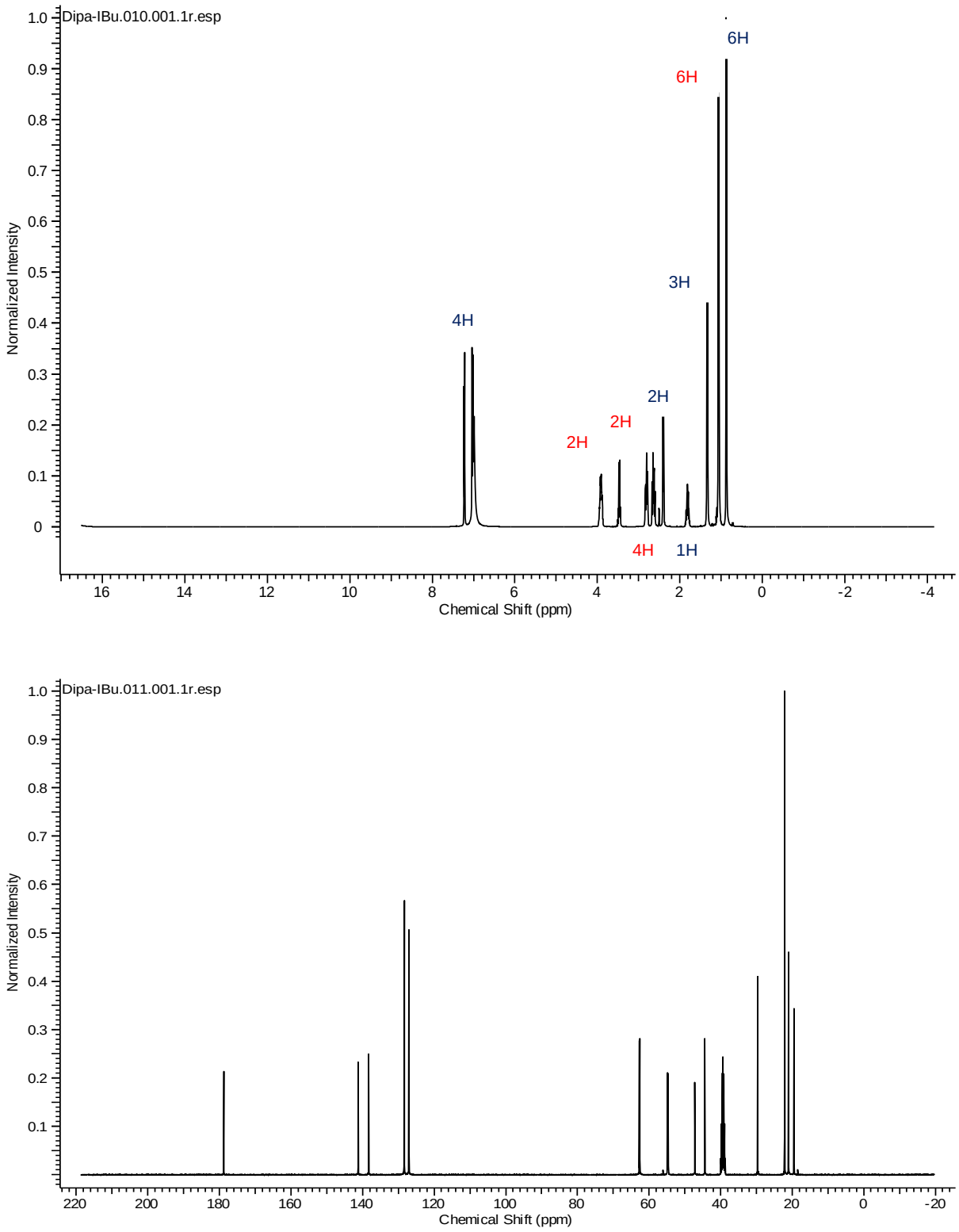


Figure 7.1 ^{1}H and ^{13}C NMR spectra of Diisopropanolamine-ibuprofen [DIPA] and [Ibuprofen] protons are represented in red and blue colours respectively

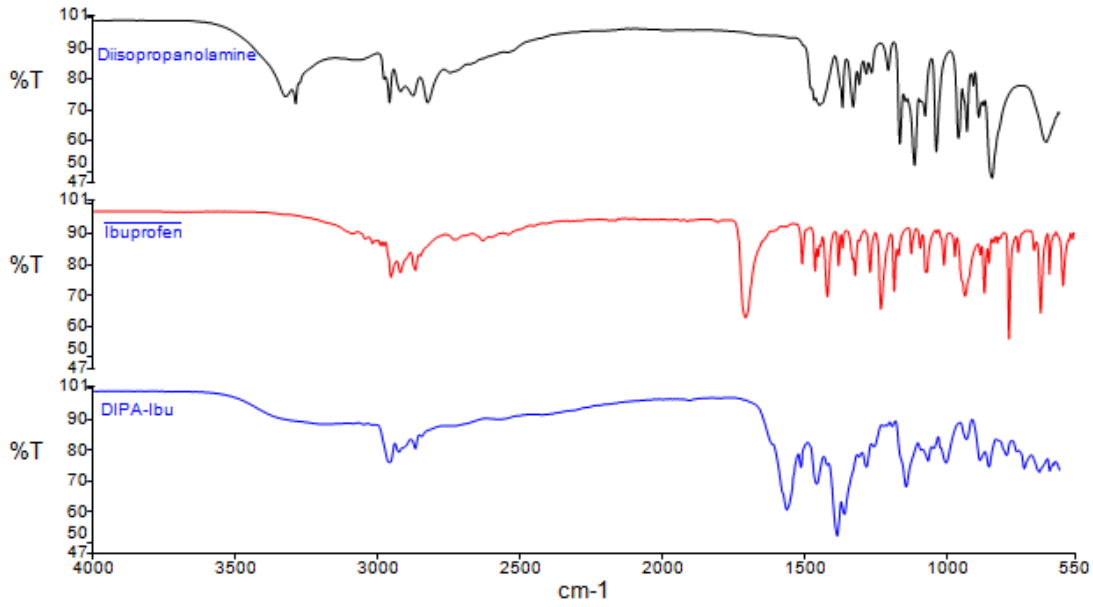


Figure 7.2 FTIR spectra of Diisopropanolamine-ibuprofen

7.2.2 Thermal behaviour

The DSC thermographs of diisopropanolamine-ibuprofen IL, free diisopropanolamine and free ibuprofen is provided in (Figure 7.3). Sharp endothermal peaks were observed at melting points: 77.14 ºC and 44.42 ºC for free ibuprofen and free diisopropanolamine respectively. These peaks were not found in diisopropanolamine-ibuprofen IL, the only thermal transition observed was a glass transition temperature at -9.11 ºC. This provides strong evidence of the formation of diisopropanolamine-ibuprofen IL, which is a salt of diisopropanolamine and ibuprofen, is different from the reacting species, and did not result in API degradation. The TGA profile of diisopropanolamine-ibuprofen IL (Figure 7.4) shows a double decomposition process with degradation temperature of 144.27 ºC.

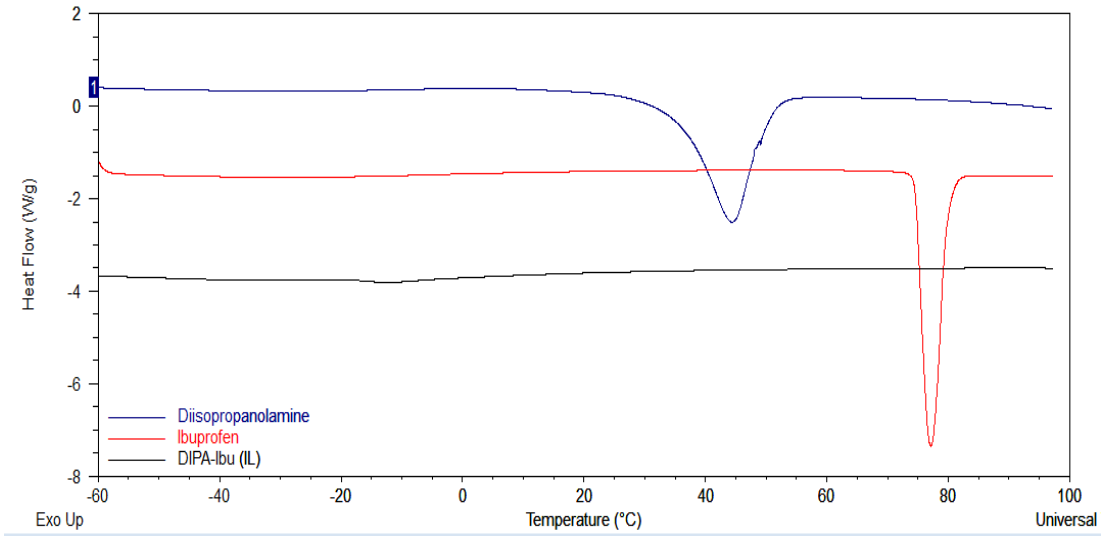


Figure 7.3 DSC thermograph of Diisopropanolamine-ibuprofen

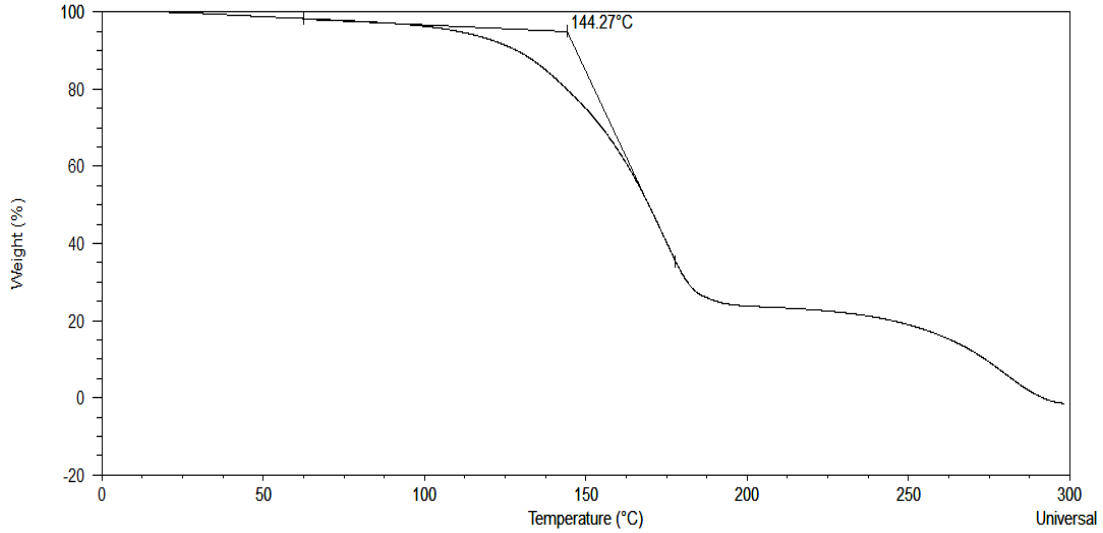


Figure 7.4 TGA profile of Diisopropanolamine-ibuprofen

7.2.3 Characterisation of IL based ibuprofen hydrogels

We employed ATR-FTIR in order to evaluate the interactions between the three components of the Ibu-DIPA-Carbopol system and to uncover the details of the mechanism of an API-IL-polymer complex formation. This technique allows to detect the formation of various types of inter- and intra-molecular interactions which take place when the aforementioned compounds interrelate with one another. As could be seen from Table 3.3 the formation of a clear gel starts with the ratio of DIPA-IBU ca. 1.5:1 indicating the completion of the gel formation and neutralisation of all the acidic groups present in the system by the ionic liquid. This suggests that the excessive amount of DIPA is required to neutralise the acidic groups of Carbopol after the reaction between Ibu and DIPA is complete. In order to verify this assumption we prepared mixtures of 2 components, namely Ibu-DIPA, Ibu-Carbopol and DIPA-Carbopol, as well as the 3 component mixtures, all with varying ratios from 1.8:1 to 0.2:1 for each component to understand which kinds of bonds occur as a result of the interactions between them.

The three component system exhibits a very complicated spectral profile (vide infra) as there are possible interactions taking place between DIPA and the carboxylic groups belonging to either Carbopol or Ibuprofen. The shift of the carbonyl vibrations to the lower wavenumbers is expected regardless the molecule it is affiliated with, and nearly identical vibration shift was detected for both DIPA-Ibu and DIPA-Carbopol systems. In view of this we initially opted for the analysis of the 2-component systems to understand what kind

of interaction can be present in these systems, which in turn would help us to understand the underlying process of a three-component gel formation.

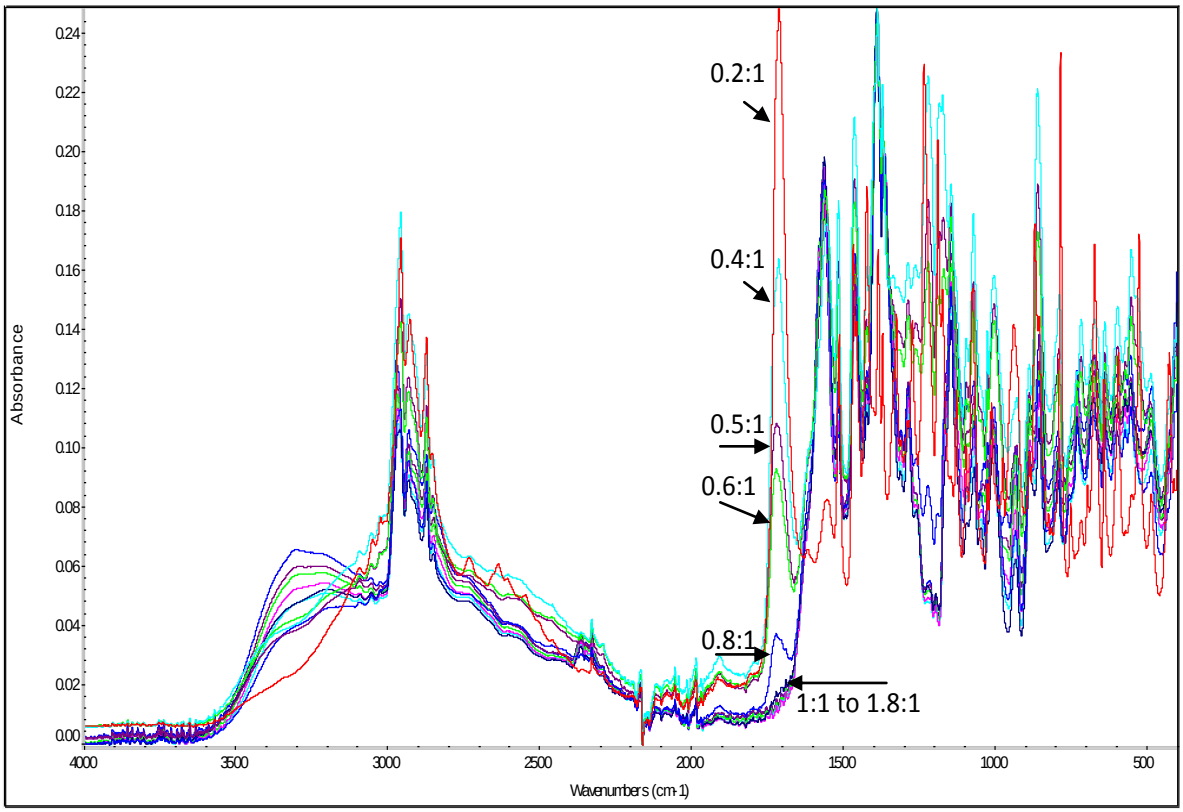


Figure 7.5 FTIR spectra showing diisopropanolamine-ibuprofen interaction

Figure 7.5 shows the interactions between DIPA and Ibu. Carbonyl stretching vibration of an ibuprofen hydrogen-bonded dimer originally appearing at 1707 cm^{-1} is expectedly shifted to lower wavenumbers (of ca. 1560 cm^{-1}) and is characteristic of an interaction of the carboxylic anion with an N-H counter-ion of aliphatic amine DIPA to form a salt (Kubota et al. 2016). Unlike reported previously, in our case even at 1:1 ratio we detected only the presence of an acid-amine salt and did not observe any co-existence of the unreacted components, which was confirmed by the complete disappearance

of the 1707 cm^{-1} vibration of Ibuprofen dimer and its reappearance as a blue-shifted bonded salt at ca. 1560 cm^{-1}

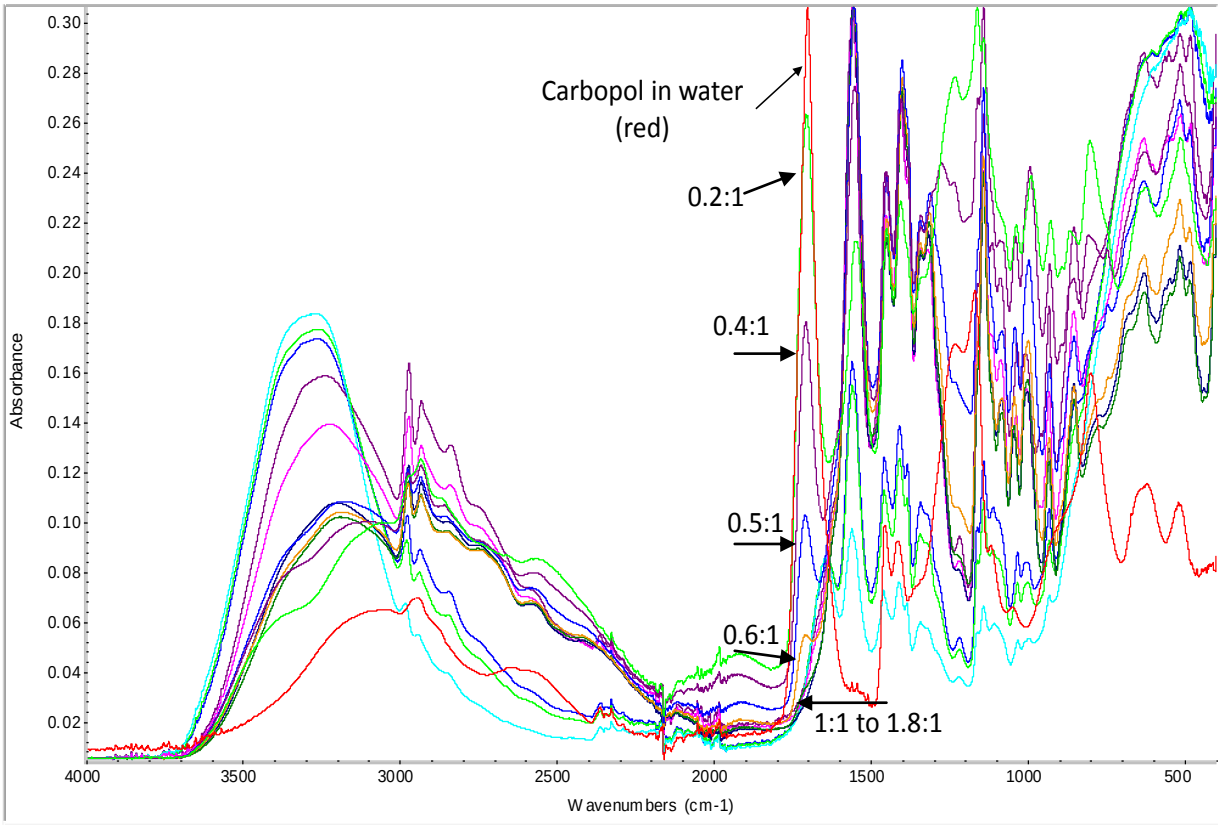


Figure 7.6 FTIR spectra showing diisopropanolamine-carbopol interaction

Figure 7.6 in turn, shows the results of interaction between Carbopol and DIPA. It is immediately apparent that neutralisation of the carboxylic groups happens gradually with the addition of DIPA, with the carbonyl stretching vibration being shifted from its original position at 1703 cm^{-1} to 1550 cm^{-1} corresponding to a COO^- functional group being ionised by DIPA (Islam et al. 2004). It can be clearly seen that the carbonyl stretching peak is diminished by the ratio of 0.6:1 and completely disappears at the higher ratios

suggesting that a virtually complete neutralisation occurs in this range of ionic liquid concentrations and thus the formation of the gel structure is achieved.

As expected, interactions between Carbopol and Ibuprofen are virtually non-existent (Figure 7.7) where the spectrum of a two-component system Ibu-Carbopol exhibits mostly peaks of ibuprofen.

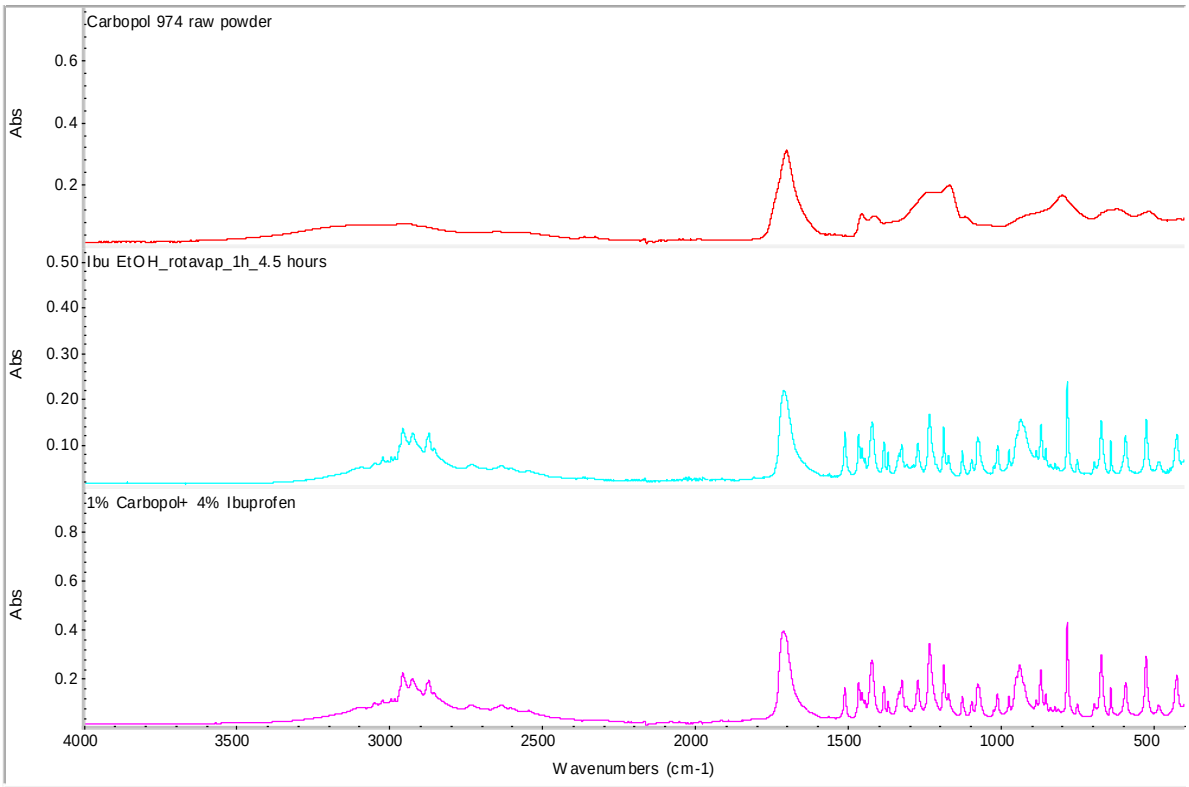


Figure 7.7 FTIR spectra showing Ibuprofen-Carbopol interaction

Having studied all possible binary combinations of Carbopol, DIPA and Ibuprofen, we attempted to evaluate what kind of interactions occur in a three-component system and whether the assumption on the suggested ratios between the components for complete neutralisation will sustain itself. We have therefore tested all the ratios of DIPA-Ibu keeping the amount of Carbopol the same for all the mixtures (Table 3.3 in section 3.3.4).

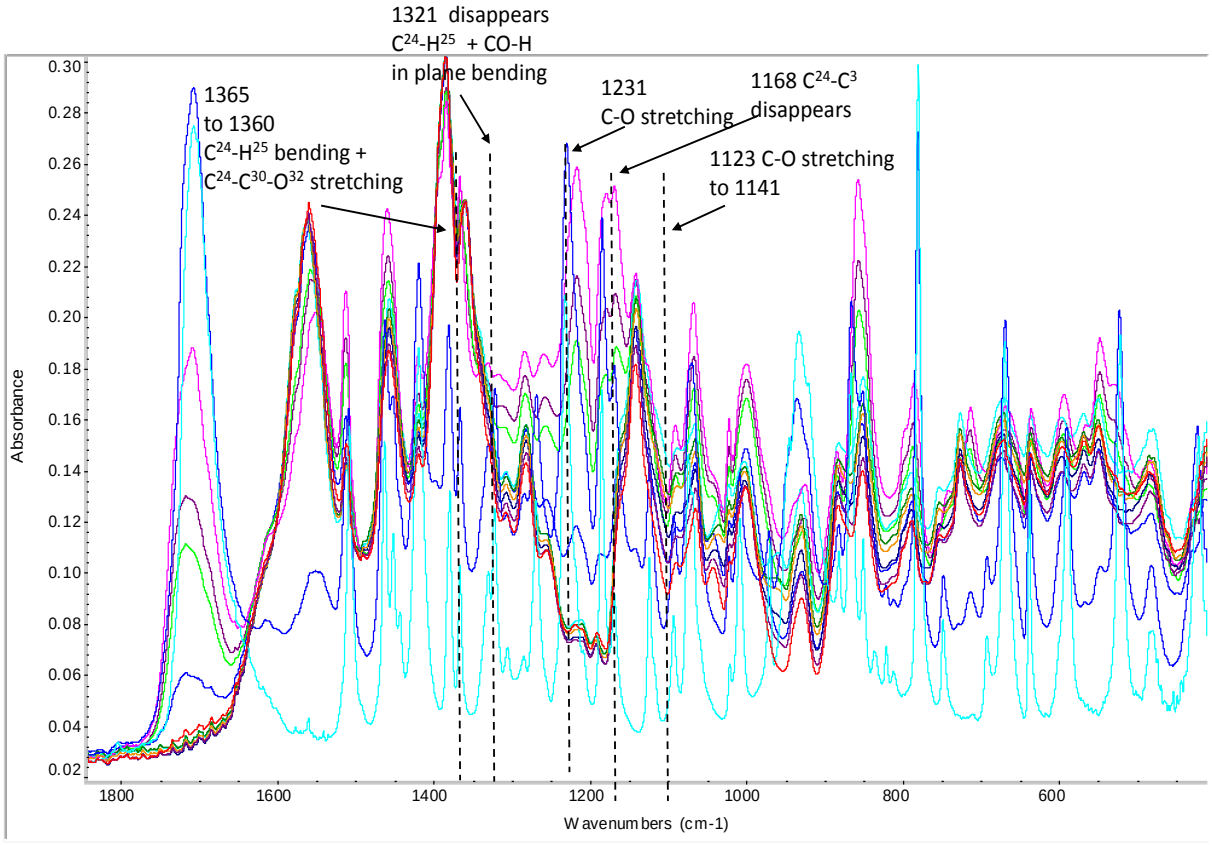


Figure 7.8 FTIR spectra showing Ibuprofen-diisopropanolamine-Carbopol interaction

As mentioned earlier it was not possible to draw meaningful conclusions, as the band shift caused by the interaction of DIPA and Carbopol overlaps with the one of DIPA and Ibu appearing in the 1550-1560 cm^{-1} region. This prompted us to concentrate on the other types of characteristic vibrations (Figure 7.8), for atom assignment see Figure 7.9.

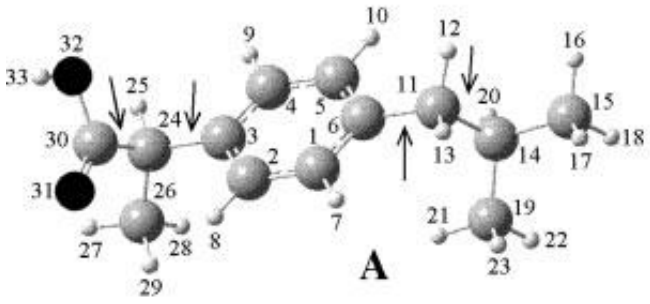


Figure 7.9 Ibuprofen atom assignments for FTIR

The propionic moiety of Ibu with the addition of DIPA becomes distorted which can be detected by the multiple changes in the peak positions of the corresponding group interactions. Thus, a C-O stretching assigned at 1123 cm^{-1} is shifted to higher wavenumbers and when more neutraliser is added a new peak appears at 1141 cm^{-1}, whereas the peaks corresponding to a C24-H25 and CO-H in plane bending at 1321-1330 cm^{-1} completely disappear, along with the peak at 1231 cm^{-1} corresponding to C-O stretching, A region at 1365 cm^{-1} corresponding to the C24-H25 bending as well as C24-C30-O32 stretching is shifted to 1360 cm^{-1}. The bond between the aliphatic C24 and aromatic C3 carbon atoms, appearing at 1168 cm^{-1} in Ibuprofen and disappearing when the amount of DIPA is enough to neutralise all the carboxylic groups (at 1:1 ratio and higher), is also affected by the interaction with an ionic liquid.

All the changes in the vibrational spectra advocate that the distortion of the aliphatic part of an Ibu molecule during the interaction with an ionic compound can be clearly detected. They also indicate that the interaction with DIPA gradually changes the shape of the part of the Ibu molecule while forming a salt, whereas Carbopol structure uncoils during neutralisation (Kemper et al. 2001) and remains unchanged after all its carboxylic groups are charge neutralised, regardless the presence of the excess of DIPA and Ibuprofen in the mix.

Overall, the information extracted from the FTIR spectra allows to theorise that the amount if IL in the mixture of DIPA:Ibu with the ratio of 1.6:1 and higher is sufficient to neutralise all Ibuprofen as well as the acidic carboxylic

groups of Carbopol, but at the same time to still maintain an acceptable slightly basic pH level to be delivered transdermally onto the skin with normal pH level lying in the broad range of 4.0 to 7.0 (Lambers et al. 2004).

7.2.4 In-vitro permeation studies

The in vitro permeation evaluation is useful for understanding drug transport across a synthetic membrane. The effect of diisopropanolamine on ibuprofen permeation across an artificial membrane was evaluated by using a Franz diffusion cell. Figure 7.10 shows the permeation of ibuprofen across the synthetic membrane. The amount of ibuprofen permeated across the artificial membrane increased with increase in concentration of diisopropanolamine. The amount of Ibuprofen permeated after application of hydrogel S1 is significantly lower than that of neat ionic liquid S4 and a hydrogel S7 containing higher amount of diisopropanolamine. This was observed irrespective of the type of membrane.

The permeation data for ibuprofen in the S1 hydrogel sample reveals less ibuprofen permeated because of its unionized free form. The FTIR data gives evidence of the presence of carbonyl group of ibuprofen for the S1 hydrogel sample, suggesting that ibuprofen is present in its unionized form. On the other hand the disappearance of the carbonyl band of ibuprofen for the S4 and S7 hydrogel samples indicates ibuprofen in ionized form. The increase in permeation rate could be attributed to the formation of neutralized ionic liquid complex of diisopropanolamine and ibuprofen. Sarveiya et al. suggested that ion pairing was responsible for higher transport of amine salts of ibuprofen over sodium salt of ibuprofen (Sarveiya et al. 2004). The permeation of Ibuprofen from S7 sample is higher among all the samples which could be

correlated to the increased ionic interactions due to formation of oligomer ionic liquid (Johansson et al. 2008). Previous literature findings also demonstrate the permeation of IL through membranes where the permeation was dependent on the nature of components (Miwa et al. 2016).

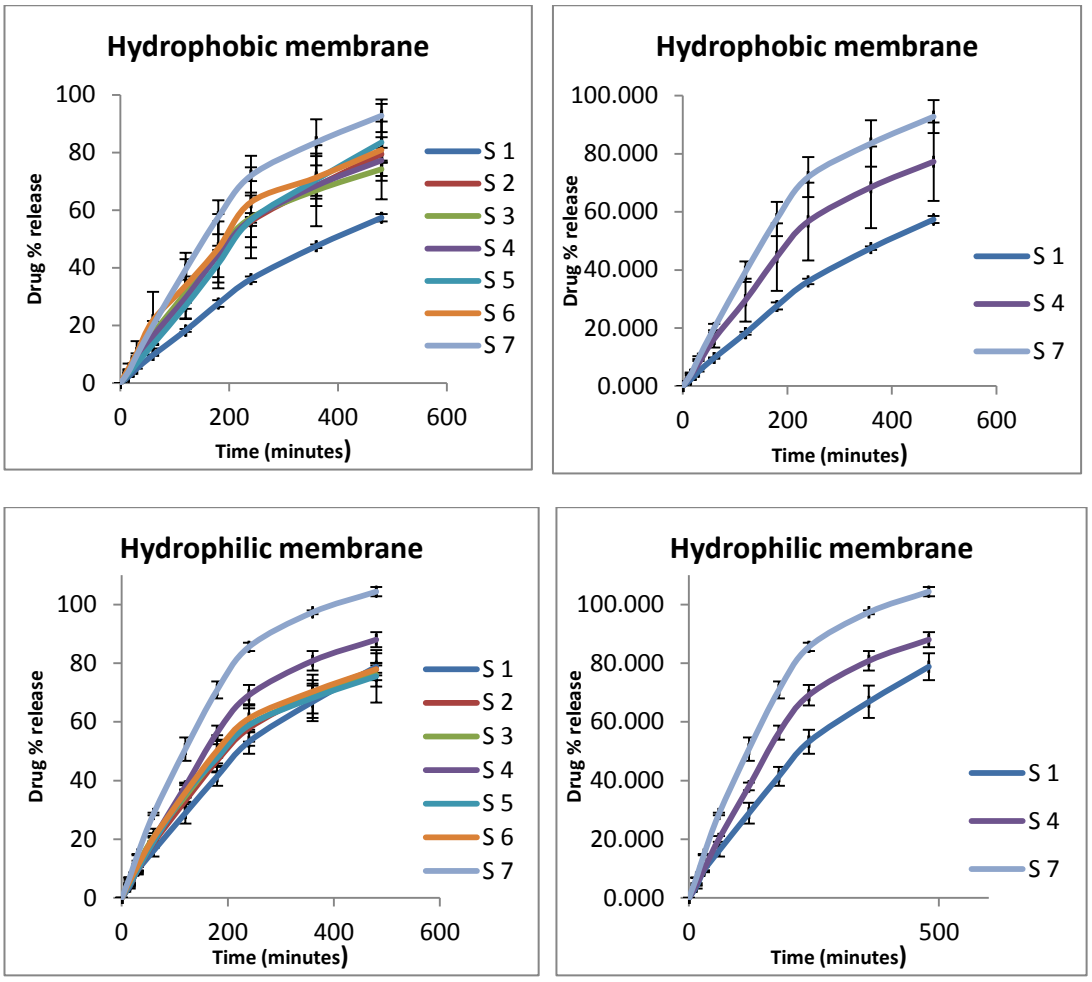


Figure 7.10 In vitro Ibuprofen permeation profile from IL based hydrogels, Refer Table 3.4 for S1-S7 formulations (Error bars - standard deviation)

7.2.5 In-vitro release studies

The drug release profiles were evaluated for all the IL based ibuprofen hydrogels in buffer solution at pH 7.2. These studies are crucial in analysing whether the formulations sustain the ibuprofen release. Difference in the release of ibuprofen from IL based ibuprofen hydrogels would be expected as the API is in the different forms. It is known that the dissolution of the drug can be affected by various factors namely polymorphism, crystallinity, particle size, solubility, viscosity (Williams et al. 2013). Therefore it is vital to compare the release profiles of ibuprofen, the model NSAID used in this study. The cumulative release percentage of ibuprofen from the IL based ibuprofen hydrogels is shown in (Figure.7.11) where it was found that the ibuprofen in the hydrogel samples S1 and S7 have similar release profiles, and both are greater than the release profile for the S4 hydrogel. This may indicate that the interaction established between ibuprofen and diisopropanolamine is significantly strong in S4 hydrogel, which is not disrupted in aqueous solution. About 80% of ibuprofen release was observed for S1 and S7 hydrogels which could be attributed to the swelling behaviour of polymer. This could be understood due to an increase in hydration degree leading to the diffusion of water into the polymeric network which helps the diffusion of the drug out of the polymeric network and into the solution. It is clear from Figure 7.11 that the release profile of ibuprofen from the S1 hydrogel sample at pH 7.4 and the S7 hydrogel sample at pH 8.7 is quite similar, although ibuprofen is in different forms, confirming that the release profile of ibuprofen is related to

the swelling behaviour of polymer. The p*Ka* of carbopol 974 polymer is 6.0 (Agarwal and Mishra 1999) and that of ibuprofen is 4.4 (Velasco et al. 2011). The degree of ionization of anionic hydrogels increases when pH is above the p*Ka* of the polymer, leading to swelling of hydrogels which finally increases the release of the drug (Gupta et al. 2002).

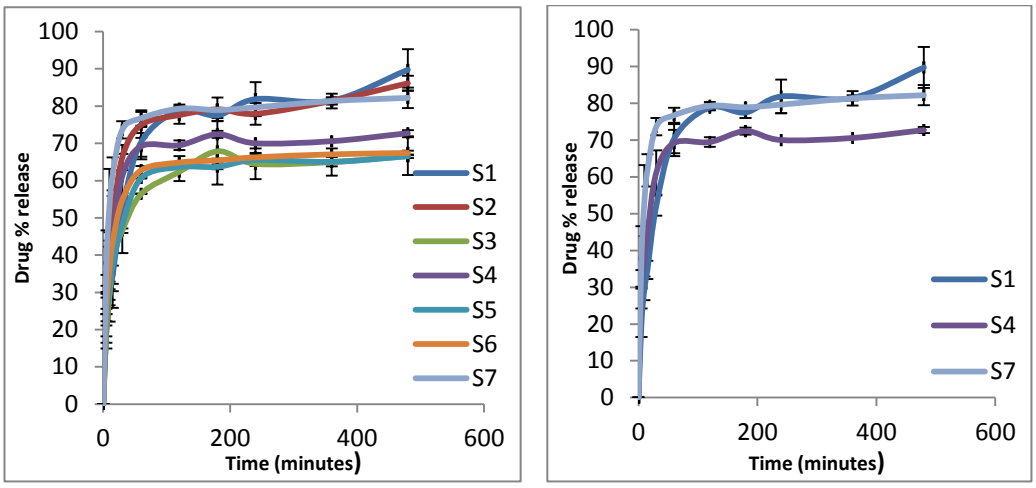


Figure 7.11 In vitro release of ibuprofen from IL based ibuprofen hydrogel, Refer Table 3.4 for S1-S7 formulations (Error bars - standard deviation)

7.3 Conclusion

The result of this study suggests the efficacy of combination of diisopropanolamine-ibuprofen IL and carbopol 974 results in situ hydrogel system for topical application. FTIR data indicate the need for a well-balanced quantity of ionic liquid and carbopol in order to achieve an effective drug-neutraliser-polymer interaction to form a hydrogel structure. The release profile of the ibuprofen from IL based ibuprofen hydrogels shows lower dissolution in its ionic liquid salt form and permeation data display a direct relationship with diisopropanolamine concentration. The efficiency of this formulation can be further studied by *Ex-vivo* experiments.

Chapter 8 Global discussion

Ionic liquids were prepared and characterised in good to excellent yields. Benzalkonium cations (benzyltriethylammonium and benzyldimethylhexadecylammonium) were combined with four different anionic drug (ibuprofen, diclofenac, salicylic acid, sulfacetamide) to generate benzalkonium based ILs. Diisopropanolamine was also coupled with ibuprofen to yield diisopropanolamine ibuprofenate IL. All these drug salts were characterised by ^{1}H, ^{13}C NMR and IR spectroscopy and subjected to evaluate their physicochemical properties and biopharmaceutical performance. Large numbers of Active Pharmaceutical Ingredient-ionic liquids (API-ILs) have been reported in the literature; to date very limited work has been conducted on their potential application in the field of topical drug delivery. The main objective of the presented work was to investigate neat API-ILs as topical drug delivery systems. To begin, a set of suitable model API-ILs were studied to address the gap in the literature.

It was illustrated in Chapter 4 that benzalkonium based NSAIDs ILs were prepared successfully by combining quaternary ammonium cations with model APIs such as ibuprofen and diclofenac. All the prepared benzalkonium based NSAIDs ILs were found to display glass transition temperature and their appearance ranged from a colourless viscous liquid to a yellow viscous liquid. The thermal decomposition temperature shows the trend of anion asymmetry and decrease in thermal decomposition temperature was observed with increase in asymmetry of anion. Interestingly the Van der Waal interactions arising due to increase in the hydrocarbon chain attached to

cation led to an increase in the thermal decomposition temperature. The conductivity data of aqueous solutions of API-ILs analysed at higher concentration suggests that at high concentration, these ILs do not show a similar trend as a salt solution. Octanol-water partition coefficient data of benzalkoium based NSAIDs ILs shows the trend of anion hydrophobicity while an increase in octanol-water partition coefficient was observed with increase in molecular weight. The results of skin deposition and permeation studies of benzalkonium based NSAIDs ILs was simply based on the partition coefficient and molecular weight measured, which suggest that [BETA] [Ibu] could be detected in the receptor compartment based on its lower molecular weight than 500 and the lower Ko/w value which is also reflected on the lower % of drug retained in the different skin layers. On the other hand, while [BTEA] [Diclo] would be expected to have a similar profile, due to the higher molecular weight the permeation was very limited. Comparatively [BDMA] [Ibu] and [BDMA] [Diclo] display very limited permeation because of high molecular volume and partition coefficient values which makes them suitable candidates for topical applications.

The study reported in chapter 5 was intended to investigate if there is a synergistic effect by combining sodium sulfacetamide and benzalkonium halides (benzyltriethylammoniuim chloride, benzyldimethylhexadecylammonium chloride) into benzalkonium sulfacetamide. The effect of the cationic counterion; benzalkonium on the benzalkonium sulfacetamide ILs physicochemical properties and biopharmaceutical performance were examined. Octanol-water partition coefficient, skin deposition and permeation data follow the similar trend as

observed in Chapter 4. The antimicrobial activity of the synthesised IL showed no synergism compared to parent molecules.

Using the idea established in Chapter 4 the study was extended to examine benzalkonium based mixed anion ILs (three component systems) with the aim of investigating the effect of ionic interaction/hydrogen bonding on the physicochemical properties and penetration behaviour via artificial membrane. All the IL with mixed anions was found to display only glass transition temperature and was found to be in the form of colourless viscous liquid to yellow viscous liquid; the only candidate displaying crystallisation temperature, melting point and glass transition temperature was M3 (benzyldimethylhexadecylammonnium ibuprofen salicylic acid) . The thermal decomposition temperature, octanol-water partition coefficient and electrical conductivity trend was found to be similar which was observed in the Chapter 4. The permeation data of ibuprofen and salicylic acid demonstrates inverse relationship with the molecular weight of the benzalkonium based mixed anion ILs. The permeation data suggest that these IL formulations exist as single entity and the penetration properties of ibuprofen and salicylic acid depends on the nature of membrane type, octanol-water partition coefficient values, molecular weights and type of interactions.

In the frame of this work ibuprofen hydrogels were developed and prepared and in-situ generation of ibuprofen-IL in the hydrogels was investigated. Ibuprofen and diisopropanolamine were combined to generate diisopropanolamine-ibuprofenate ionic liquid then the prepared IL was incorporated in the Carbopol 974 water dispersion to form hydrogels. FTIR analysis was performed in order to understand the interaction of ibuprofen-IL

hydrogels. In order to improve the appearance, pH, diffusion and permeation through artificial membrane the ratio of diisopropanolamine was varied keeping the amount of Carbopol 974 and ibuprofen constant. Drug diffusion studies were conducted to evaluate the liberation of ibuprofen from the vehicle and the diffusion study was performed via synthetic membrane using Franz diffusion cell. It could be assessed, that permeation of ibuprofen follows direct relationship with the concentration of base which might arise due to increase in the ionic interaction between ibuprofen and diisopropanolamine.

Chapter 9 Conclusions and future

In summary, this thesis illustrates the tunable strategy of the IL which provides a potential tool to tailor the physical, chemical and biological properties which allows generating topical drug delivery and improved pharmaceutical application. Some positive results for topical application of neat ILs have been reported in this dissertation.

9.1 Conclusion

1. Four benzalkonium based NSAID ILs were synthesised and investigated for their physicochemical and biopharmaceutical performance. The results suggest that all the prepared ILs are suitable for topical application.
2. Benzalkonium-sulfacetamide ILs were evaluated to look into if there is any synergistic effect between benzalkonium cation and sulfacetamide anion. The data indicates no synergism between both components.
3. The transport properties of benzalkonium based mixed anion ILs was found to be dependent on the octanol-water partition coefficient, nature of the permeant and nature of artificial membrane used to study.
4. Ibuprofen IL hydrogel formulation was investigated to determine the in-situ formation of ibuprofen IL in ibuprofen topical gel. FTIR-ATR was found to be the potential tool to understand the interaction between the components of hydrogel formulation.

9.2 Suggested Future work

1. This work looked at only small sub-set of ILs by investigating the change of alkyl chain length and the effect of anion on the topical applications of NSAIDs. It would be interesting to carry out the same analysis to determine the effect of a series of alkyl side chain of different cation.
2. It would be of interest to look into the effect of series of alkyl chain length on cation to understand the synergistic effect of benzalkonium sulfacetamide ILs.
3. IL formation dramatically enhances the transport property of drug. The overall mechanism depends on the counterion and was not well studied; therefore the dissociation behaviour of ion pair aggregates before and after penetrating the membrane would be an interesting area to look into.
4. It was shown here that IL modular strategy can be utilised to form hydrogels. It would be interesting to look into the rheological properties and penetration behaviour of API by using different anionic polymers. The effect of different concentration of polymers the diffusion properties of API could also be investigated.

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Appendix

Contents

1. **Figure.S1, S2** ^{1}H and ^{13}C NMR, FTIR spectra of triisopropanolamine-ibuprofen IL
2. **Figure S3** and Solubility determination of Ibuprofen ILs

1. NMR and FTIR characterization data

Triisopropanolamine-ibuprofen:

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.87 (6 H), 0.98 - 1.10 (m, 9 H), 1.36 (3 H), 1.82 (1 H), 2.35 - 2.44 (2 H), 2.46 - 2.57 (m, 6 H), 3.61 (1 H), 3.70 - 3.83 (3 H), 6.07 (3 H), 7.00 - 7.12 (2H), 7.14 - 7.27 (2 H)

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 18.61, 20.29, 20.75, 21.07, 22.06, 29.60, 38.77, 38.98, 39.18, 39.40, 39.61, 44.35, 44.89, 62.82, 63.33, 63.81, 64.10, 64.54, 64.66, 127.01, 128.70, 138.94, 139.15, 175.95

IR (ν_{max} cm^{-1}): 2965.54, 2929.32, 1719.42, 1567.26, 1511.75, 1458.04, 1216.21, 1140.62, 1059.45, 1004.64, 854.35, 785.92

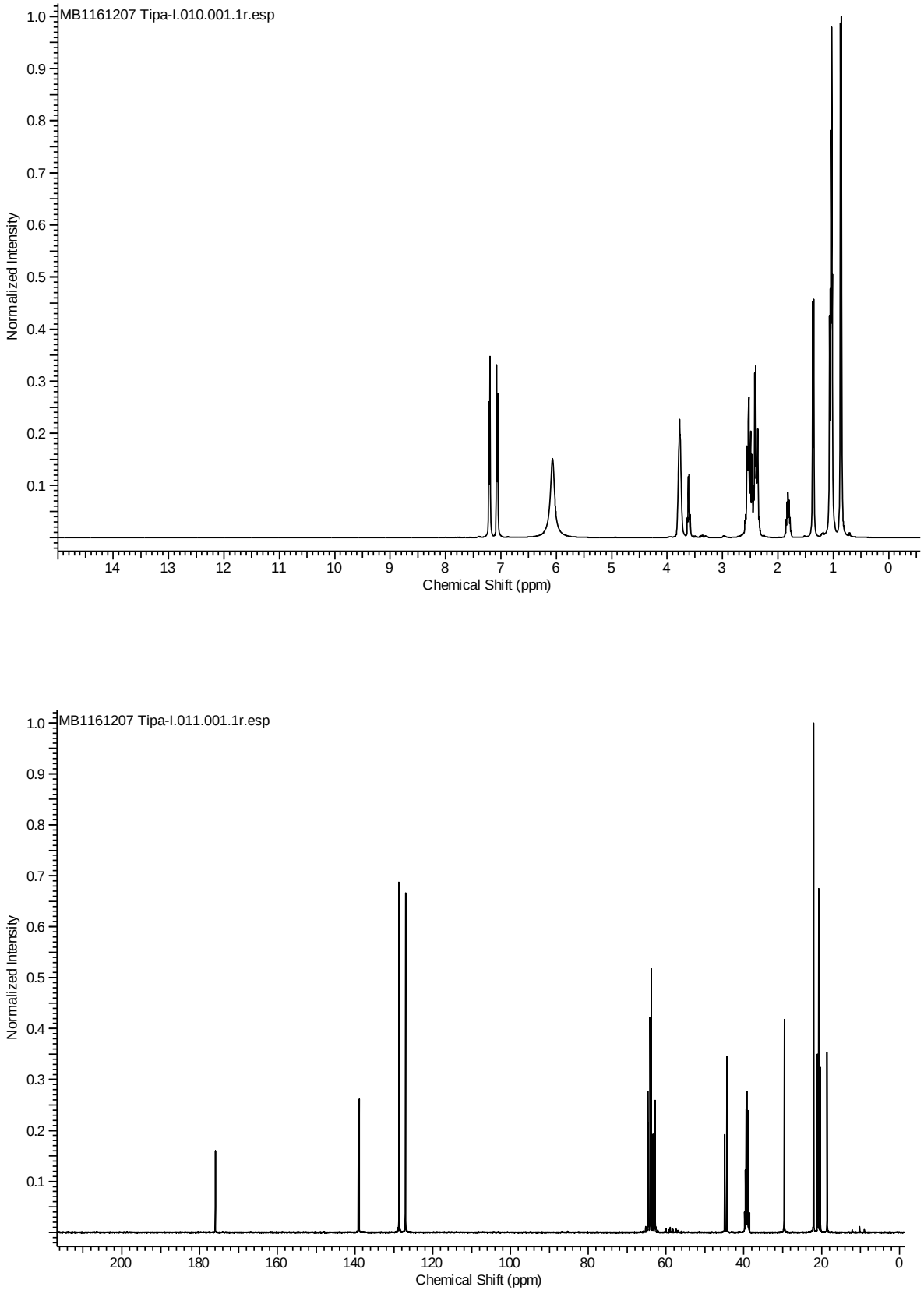

Figure.S1 ^{1}H and ^{13}CNMR spectra of triisopropanolamine-ibuprofen

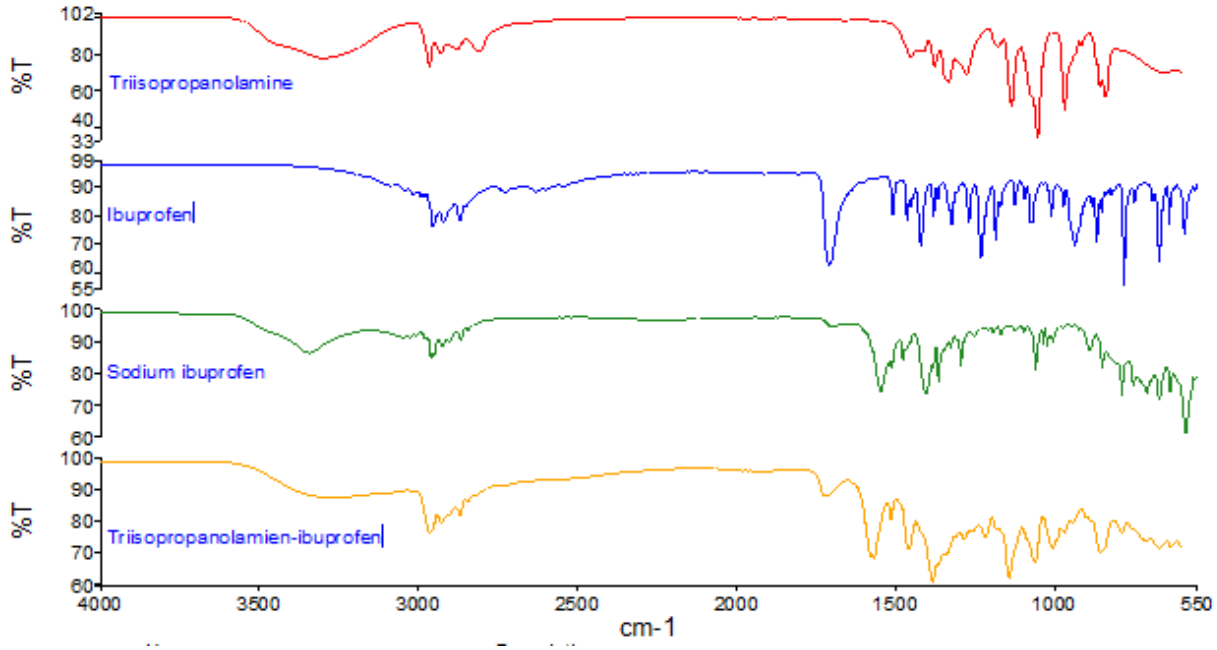

Figure.S2 FTIR spectra of triisopropanolamine-ibuprofen

2. Solubility determination of ILs

1. Equimolar ratio of diisopropanolamine-ibuprofen, triisopropanolamine-ibuprofen, triethanolamine-ibuprofen was charged in 10 ml vial and stirred at 80 – 90 °C for 30-40 minutes. Then the prepared IL mixture was used for solubility studies.

Table.1 Solubility determination of ILs

IL / DI Water (ml)	Solubility determination		
	*Dipa-ibu 266 : 412 (mg)	*Tipa-ibu 382 : 412 (mg)	*TEA-ibu 298 : 412 (mg)
0.1	Colourless solution	Yellowish solution	Brownish solution
0.5	Colourless solution	Yellowish solution	Brownish solution
1	Colourless solution	Yellowish solution	Brownish solution
2	Colourless solution	Yellowish solution	Brownish solution
5	Colourless solution	Yellowish solution	Brownish solution
10	Colourless solution	Yellowish solution	Brownish solution
20	Colourless solution	Turbid solution	Turbid solution
22	Colourless solution	Precipitation	Precipitation
200	Colourless solution	-	-

*DIPA-Ibu - Diisopropanolamine-ibuprofenate

*TIPA-Ibu - Triisopropanolamine-ibuprofenate

*TEA-Ibu - Triethanolamine-ibuprofenate

TEA-Ibu Tipa-Ibu Dipa-Ibu

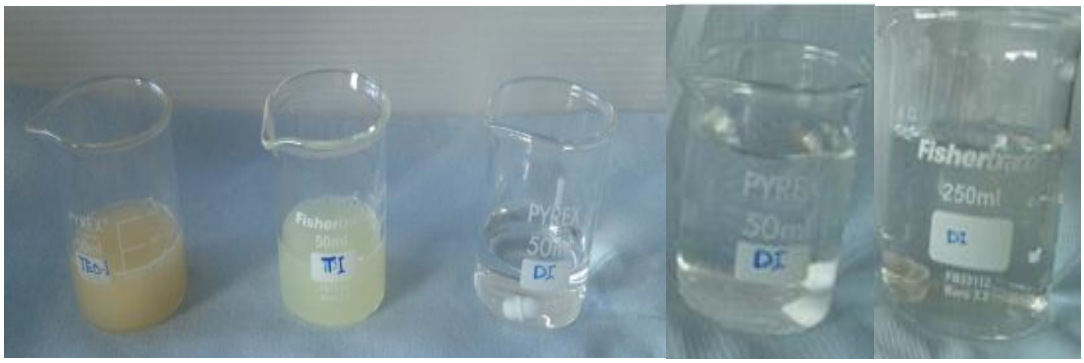

Figure S3 Solubility determination of ILs